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DERWENT-WEEK: 199716

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TITLE: Drug compsn. which is biodegradable  
and decomposed to  
aminoacid in body - contains  
tri:peptide contg. proline  
or di:peptide comprising arginine and  
glutamine as  
artificial sweetener.

PATENT-ASSIGNEE: MITSUI TOATSU CHEM INC[MITK]

PRIORITY-DATA: 1995JP-0124900 (May 24, 1995)

PATENT-FAMILY:

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APPL-DATE		
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INT-CL (IPC): A61K038/00, C07K005/072 , C07K005/097

ABSTRACTED-PUB-NO: JP 09040577A

BASIC-ABSTRACT:

Drug compsn. contains a tripeptide of formula Pro-A-B (A = Phe, Lys, Asn, Tyr or Thr; B = Pro or Trp), C-D-Pro (C = Tyr, Glu or Pro; D = Asn, Ser, Arg or Tyr), Thioproline-Thr-Trp or a dipeptide of Glu-Arg, or their salts.

ADVANTAGE - The drug is biodegradable and is decomposed to amino acid in a living body and metabolised. It has no side effects.

In an example, Pro-Phe-Pro was synthesised. 2.7 g BOC-Phe,

2.4 g ProOBzl HCl  
and 1.5 g HOBt were dissolved in 30 ml DMF. 1.7 g WSCD HCl  
in 10 ml DMF was  
added to it. 1.8 ml TEA was added and the mixture was  
stirred at room temp.  
overnight. It was filtered and the filtrate was conc. in  
vacuo and 300 ml  
ethyl acetate was added and washed with 4% Na bicarbonate  
in 10% NaCl, 10%  
NaCl, 0.4M citric acid in 10% NaCl and 10% NaCl. The ethyl  
acetate layer was  
dried on anhydrous Na sulphate and conc. in vacuo and  
dried. 40 ml 4N  
HCl/dioxane and 4 ml thioanisole were added and reacted for  
2 hrs. while  
ice-cooled. It was conc. under an addition of dioxane and  
diethyl ether,  
respectively, twice. 1.5 g Z-Pro and 0.84 g HOBt were  
added and dissolved in  
30 ml DMF. 1.2 g WSCD HCl in 10 ml DMF was added and  
reacted for 1.5 hrs. at  
room temp. and then at 4 deg.C overnight. The product was  
filtered and the  
filtrate was washed, concd. and purified. It was treated  
with hydrogen gas in  
the presence of Pd/C and purified to give amorphous  
colourless product.

CHOSEN-DRAWING: Dwg.0/0

TITLE-TERMS: DRUG COMPOSITION BIODEGRADABLE DECOMPOSE  
AMINOACID BODY CONTAIN  
TRI PEPTIDE CONTAIN PROLINE DI PEPTIDE COMPRISE  
ARGININE GLUTAMINE  
ARTIFICIAL SWEET

DERWENT-CLASS: B04 B05 D13 E16

CPI-CODES: B04-C01A; B07-D03; B10-A17; D03-H01A; E07-D03;  
E10-A17;

CHEMICAL-CODES:

Chemical Indexing M2 \*01\*

Fragmentation Code

D011 D601 F011 F012 F019 F423 F499 G010 G013 G100  
H100 H181 H211 H401 H441 H481 J0 J013 J014 J1  
J111 J171 J3 J311 J371 J372 M280 M312 M313 M315  
M321 M322 M332 M343 M349 M371 M381 M391 M392 M412  
M413 M510 M511 M521 M522 M530 M531 M540 M630 M640  
M650 M781 M903 M904 Q222

Markush Compounds  
199716-16601-U

Chemical Indexing M3 \*01\*

Fragmentation Code

D011 D601 F011 F012 F019 F423 F499 G010 G013 G100  
H100 H181 H211 H401 H441 H481 J0 J013 J014 J1  
J111 J171 J3 J311 J371 J372 M280 M312 M313 M315  
M321 M322 M332 M343 M349 M371 M381 M391 M392 M412  
M413 M510 M511 M521 M522 M530 M531 M540 M630 M640  
M650 M781 M903 M904 Q222

Markush Compounds  
199716-16601-U

Chemical Indexing M2 \*02\*

Fragmentation Code

F011 F012 F019 F423 F499 G013 G019 G100 H100 H181  
H2 H211 H401 H402 H441 H442 H481 J0 J013 J014  
J1 J111 J171 J3 J311 J371 J372 J373 L250 M280  
M312 M313 M314 M321 M322 M332 M343 M349 M371 M381  
M391 M392 M413 M510 M521 M522 M530 M531 M532 M540  
M630 M640 M650 M781 M903 M904 Q222

Markush Compounds  
199716-16602-U

Chemical Indexing M3 \*02\*

Fragmentation Code

F011 F012 F019 F423 F499 G013 G019 G100 H100 H181  
H2 H211 H401 H402 H441 H442 H481 J0 J013 J014  
J1 J111 J171 J3 J311 J371 J372 J373 L250 M280  
M312 M313 M314 M321 M322 M332 M343 M349 M371 M381  
M391 M392 M413 M510 M521 M522 M530 M531 M532 M540  
M630 M640 M650 M781 M903 M904 Q222

Markush Compounds  
199716-16602-U

Chemical Indexing M2 \*03\*

Fragmentation Code

D011 D601 F014 F710 H4 H401 H481 H8 J0 J013  
J1 J171 J3 J311 J371 M280 M312 M313 M321 M331  
M332 M343 M349 M371 M381 M391 M412 M511 M521 M530  
M540 M630 M640 M650 M781 M903 M904 Q222

Markush Compounds  
199716-16603-U

Chemical Indexing M3 \*03\*

Fragmentation Code

D011 D601 F014 F710 H4 H401 H481 H8 J0 J013  
J1 J171 J3 J311 J371 M280 M312 M313 M321 M331  
M332 M343 M349 M371 M381 M391 M412 M511 M521 M530

M540 M630 M640 M650 M781 M903 M904 Q222  
Markush Compounds  
199716-16603-U

Chemical Indexing M2 \*04\*

Fragmentation Code

H1 H100 H181 J0 J013 J1 J172 J3 J371 K0  
L2 L250 M280 M313 M314 M321 M332 M343 M349 M381  
M392 M416 M620 M630 M640 M650 M781 M903 M904 Q222  
Markush Compounds  
199716-16604-U

Chemical Indexing M3 \*04\*

Fragmentation Code

H1 H100 H181 J0 J013 J1 J172 J3 J371 K0  
L2 L250 M280 M313 M314 M321 M332 M343 M349 M381  
M392 M416 M620 M630 M640 M650 M781 M903 M904 Q222  
Markush Compounds  
199716-16604-U

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(54) 【発明の名称】 トリペプチド、ジペプチドを含有する医薬組成物

## (57) 【要約】

【課題】 制癌効果を有する特定のアミノ酸配列を有するトリペプチド、ジペプチド或いはその誘導体を含有する医薬組成物を提供する。

【解決手段】 Pro-A-B (ただしA=Phe, Lys, Asn, Tyr, Thr; B=Pro, Trpを示す。)で示されるトリペプチド、またはそれらの生理的に認められた塩を有効成分として含有する医薬組成物、C-D-Pro (ただしC=Tyr, Glu, Pro; D=Asn, Ser, Arg, Tyrを示す)で示されるトリペプチド、およびGlu-Argで示されるジペプチドまたはそれらの生理的に認められた塩を有効成分として含有する医薬組成物。

【効果】 本発明の医薬組成物はその有効成分がペプチドであり、生体内でアミノ酸に分解され代謝される。そのため、本発明の医薬組成物は生体に投与した場合に副作用を起こすことが極めて少なく、医薬品として有効である。

## 【特許請求の範囲】

【請求項1】 Pro-A-B（ただしA=Phe, Lys, Asn, Tyr, Thr; B=Pro, Trpを示す。）で示されるトリペプチド、またはそれらの生理的に認められた塩を有効成分として含有する医薬組成物。

【請求項2】 C-D-Pro（ただしC=Tyr, Glu, Pro; D=Asn, Ser, Arg, Tyrを示す）で示されるトリペプチド、またはそれらの生理的に認められた塩を有効成分として含有する医薬組成物。

【請求項3】 Thioproline-Thr-Trpで示されるトリペプチド、またはそれらの生理的に認められた塩を有効成分として含有する医薬組成物。

【請求項4】 Glu-Argで示されるジペプチド、またはそれらの生理的に認められた塩を有効成分として含有する医薬組成物。

【請求項5】 トリペプチドのN末端がアシル化された誘導体、またはそれらの生理的に認められた塩であることを特徴とする特許請求の範囲第1項～4項の何れか1項に記載の医薬組成物。

【請求項6】 トリペプチドのC末端がアミド化された誘導体、またはそれらの生理的に認められた塩であることを特徴とする特許請求の範囲第1項～4項の何れか1項に記載の医薬組成物。

【請求項7】 制癌剤であるところの特許請求の範囲第1～6項に記載の医薬組成物。

## 【発明の詳細な説明】

## 【0001】

【発明の属する技術分野】本発明は制癌剤として有用なトリペプチド或はジペプチドを含有する医薬組成物に関するものである。

## 【0002】

【従来の技術】医薬品としてのトリペプチド及びその誘導体を利用することに関しては、トロンビン活性の阻害剤の研究が知られている（ジャーナル メディカル ケミストリー: vol. 37, p. 2122, 1994年 同: vol. 36, p. 300, 1993年）。また、比較的短いペプチド及びその誘導体の医薬品としての開発研究は、例えば特表平4-502154号公報、特表平4-502306号公報、特表平4-502308号公報、特表平4-502309号公報等に見られる。これらの研究では、そのペプチドのアミノ酸配列にプロリン基が含まれ、配列の長さはアミノ酸の数が5残基以上である。これらのペプチドは腫瘍壊死因子（TNF）から誘導されたTNF改良ペプチドとも言えるものである。このように、短いペプチドを医薬品として利用する研究は幾つか進められていた。短いペプチドは細胞内に取り込まれやすいことが十分考えられ、尚且つそれらは生体内で分解されて無害なアミノ酸となるため、生体への投与に対する副作用はほとんど生じないものと考えられる。故にそれら短いペプチドは癌を始め他の病気の治療薬として将来的に有望な薬剤となりうると考えられる。しかし、短いペプチ

ドの制癌剤としての利用に関してはこれまで全く知られていなかった。

## 【0003】

【発明が解決しようとする課題】本発明者らは、癌細胞の増殖メカニズムを調べていくうちにその重要な代謝経路を決定しているのは蛋白質-蛋白質相互作用であることを知るに至った。一般的に、蛋白質と蛋白質との結合には蛋白質自体の複雑な構造が関与しているものと考えられているが、プロテアーゼとそのインヒビターとの結合ドメインの詳細な研究の結果から、蛋白質の結合に関与しているのは大きな分子量の蛋白質のごく一部の短いペプチド鎖であることがわかった。以上のことから癌細胞の増殖に關与する蛋白質の結合ドメインに相当するペプチドを細胞内に共存させれば、該蛋白質と他の蛋白質との結合を阻止することができ、その結果該蛋白質の機能を抑制することが可能であるため、その様な短いペプチドは制癌剤として有用であると考えられた。本発明の目的は、制癌効果を有する短いペプチドを提供することであり、さらに具体的には、本発明の目的は特定のアミノ酸配列を有するトリペプチド、ジペプチド或いはそれらの誘導体を含有する医薬組成物を提供することにある。本発明の他の目的は特定のアミノ酸配列を有するトリペプチド、ジペプチド或いはその誘導体の生理的に認められた塩を含有する医薬組成物を提供することにある。

## 【0004】

【課題を解決するための手段】本発明者らは有望なトリペプチドのアミノ酸配列として種々の癌遺伝子産物、例えば K-Sam, Yes, Ret, Kit, Fms, ErbB, Met, Ros, Sea, Trk, Src, Fgr, Fyn, Lyn, Lck, Hck, Abl, Arg等や、さらにはサークホモロジー(SH)ドメインと呼ばれる配列を有する癌遺伝子産物のコンセンサス配列を検索し、結合ドメインに相当するトリペプチド等の研究を通じて蛋白質の結合に関与する配列を予測した。その結果、一定のアミノ酸配列を有するトリペプチド等が癌細胞の増殖を阻害する効果を有することを見だし、本知見に基づき本発明を完成するに至った。

【0005】即ち、本発明はPro-A-B（ただしA=Phe, Lys, Asn, Tyr, Thr; B=Pro, Trpを示す。）で示されるトリペプチド、またはそれらの生理的に認められた塩を有効成分として含有する医薬組成物、Thioproline-Thr-Trp（以下thioPro又はP<sup>+</sup>）で示されるトリペプチド、またはそれらの生理的に認められた塩を有効成分として含有する医薬組成物、Glu-Argで示されるジペプチド、またはそれらの生理的に認められた塩を有効成分として含有する医薬組成物、C-D-Pro（ただしC=Tyr, Glu, Pro; D=Asn, Ser, Arg, Tyrを示す）で示されるトリペプチド、またはそれらの生理的に認められた塩を有効成分として含有する医薬組成物を提供するものである。

## 【0006】

【発明の実施の形態】本発明に係わるトリペプチドのアミノ酸配列としては例えばPro-Phe-Pro、Pro-Lys-Pro、Pro-Tyr-Pro、Tyr-Ser-Pro、Glu-Arg-Pro、Pro-Tyr-Trpなどである。本発明に係わるジペプチドのアミノ酸配列としては例えばGlu-Argなどである。本発明においては、上記トリペプチド、ジペプチドのN末端のアミノ酸のアシル化されたもの、C末端のアミノ酸のアミド化されたもの、および両端が上記のごとく修飾されたものも利用される。本発明のトリペプチド、ジペプチドのN-アシル化誘導体としてはホルミル基、アセチル基、アリールカルボニル基、芳香族カルボニル基誘導体であり、C-アミド化誘導体としてはアミノ基、アルキルアミノ基、芳香族アミノ基誘導体をあげることができる。本発明のトリペプチド、ジペプチド及びその誘導体の生理的に認められた塩としては塩酸、クエン酸、リン酸、酒石酸、乳酸、酢酸、ギ酸、フマル酸、マレイン酸、コハク酸などがあげられる。

【0007】本発明に係わるトリペプチド、ジペプチド及びその誘導体の合成は、例えば日本生化学会編集統生化学実験講座2 蛋白質の化学(下)に記載のあるように固相法や液相法のいずれの方法によっても容易に合成することができる。本発明に係わるトリペプチド、ジペプチド及びその誘導体の精製は、シリカゲルカラムクロマトグラフィー、イオン交換カラムクロマトグラフィー等通常の方法で行うことができる。溶出液にも限定はなく水、メタノール、クロロホルム等通常の有機溶媒を用いることができる。本発明に係わるトリペプチド、ジペプチド及びその誘導体の合成を更に詳しく述べれば、まずカルボキシ基をベンジル基(Bzl基)で保護した市販品のアミノ酸とを1-エチル-3-(3-ジメチルアミノプロピル)-カルボジイミド塩酸塩(WSCD HCl)やジシクロヘキシルカルボジイミド(DCCD)等の縮合剤を用いて縮合しジペプチドを得る。本発明のトリペプチド、ジペプチドの縮合反応に用いる溶媒としてはN,N-ジメチルホルムアミド(DMF)、ジメチルスルホキシド(DMSO)、アセトニトリル、1,4-ジオキサン、テトラヒドロフラン(THF)ならびにこれらの混合物が好適である。トリペプチド、ジペプチドの保護基の除去はトリフルオロメタンスルホン酸法(TMSFA法)やパラジウム/炭素を触媒とした水素接触還元法或は液化フッ化水素(HF)を用いることができる。水素接触還元法で用いる溶媒としては一般的に用いられているもので問題はない。メタノール(以下MeOHと略記することもある)、酢酸あるいはこれらの混合物が好適である。各合成ステップにおける反応進行や純度のチェックには薄層クロマトグラフィー(TLC)の一般的な方法を用いることができる。展開溶媒はクロロホルム-メタノール系、n-ブタノール-酢酸-ピリジン-水(4:1:1:2)系を用い、スポットの確認は臭化水素(HBr)-ニンヒドリン法を用いることができる。

【0008】本発明の医薬組成物は、以上のようにして得られたトリペプチド、ジペプチド及びその誘導体、またはそれらの生理的に認められた塩を有効成分として含有する以外特に制約はないが、化合物の製剤化に際して通常使用される添加剤を含んでもよい。本発明の医薬組成物は、優れた活性を有する制癌剤として使用され得る。適応される癌種は、白血病、骨肉腫、乳癌、卵巣癌、胃癌、大腸癌、肺癌等である。本発明の医薬組成物の投与方法は、投与対象の症状により当然異なるが、成人1人1日あたり0.01~10gを1回または数回に分割して投与する。尚、本発明の医薬組成物の副作用は通常の使用程度では何等問題がない。

【0009】制癌効果の判定は種々の癌遺伝子で癌化した培養細胞株の培養液に本発明のトリペプチド及びその誘導体またはこれらの生理的に認められた塩を加え、通常の条件下に培養することによって細胞の形態、増殖率、生存率などの変化を観察することができる。例えば癌細胞の増殖率抑制効果測定には3-[4,5-ジメチルチアゾール-2-イル]-2,5ジフェニルテトラゾリウム臭素塩法(MTT法)を用いると便利である。癌細胞株としては例えばv-Ha-ras、Ki-ras、v-src、c-erbB-2、v-mos、SV40などの癌遺伝子で癌化したNIH3T3線維芽細胞や3Y1ラット線維芽細胞などの培養細胞株をあげることができる。以下に実施例により本発明を具体的に説明するが、本発明はこれによって限定されるものではない。

#### 【0010】

##### 【実施例】

##### 実施例 1 [Pro-Phe-Pro(PFP)の合成]

Boc-Phe 2.7g (10ミリモル)、ProOBzl HCl 2.4g (10ミリモル)、1-ヒドロキシベンゾトリアゾール(HOBT) 1.5g (11ミリモル)をDMF 30mlに溶解し、氷冷攪拌下にDMF 10mlに懸濁したWSCD HCl 1.7g (11ミリモル)を滴下した。トリエチルアミン(TEA) 1.8mlを加え、万能pH試験紙でpH7~8であることを確認した後、室温で終夜攪拌した。析出した沈澱を濾過して除き、母液を減圧濃縮した後、酢酸エチル(EtOAc) 300mlを加え、4%炭酸水素ナトリウム(NaHCO<sub>3</sub>)の10%食塩(NaCl)水溶液、10%NaCl水溶液、0.4Mクエン酸の10%NaCl水溶液、10%NaCl水溶液の順に洗浄した。EtOAc層に無水硫酸ナトリウム(Na<sub>2</sub>SO<sub>4</sub>)を加えて脱水後、溶媒を減圧留去し、残渣をデシケーター中で減圧乾固した。この乾固物に4N 塩酸/ジオキサン 40ml、チオアニソール 4mlを加え、氷冷下2時間反応した。これを減圧濃縮した後、ジオキサン 50mlを加え、濃縮する操作を2回繰り返す。同様にジエチルエーテル(Et<sub>2</sub>O)を加え濃縮する操作を2回繰り返す。これを減圧乾固した乾固物に、Z-Pro 1.5g (6.2ミリモル)、HOBT 0.84g (6.8ミリモル)を加え、DMF 30mlに溶解した。氷冷攪拌下、DMF 10mlに懸濁したWSCD HCl 1.2g (6.8ミリモル)を滴下した。TEA 1.6mlでpH7~8に調製した後、室温で1.5時間反応させた。さらに4℃で終夜反応さ

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せた後、析出した沈澱を濾過して除き、母液を減圧濃縮した。EtOAc 300mlを加え、4%NaHCO<sub>3</sub>の10%NaCl水溶液、10%NaCl水溶液、0.4Mクエン酸の10%NaCl水溶液、10%NaCl水溶液の順に洗浄した。EtOAc層に無水Na<sub>2</sub>SO<sub>4</sub>を加えて脱水後、溶媒を減圧留去し、残渣をデシケーター中で減圧乾固した。乾固物を少量のメタノール(MeOH)に溶解し、セファデックス LH-20カラム(内径3cm×長さ55cm)にて分離精製した。同溶媒で溶出し、1フラクションあたり5分(約8ml)で分画した。各フラクションについて TLC (キーゼルゲル60F<sub>254</sub>展開溶媒には、クロロホルム-MeOHの系を用いた。)で確認後、目的物を含む画分を集め減圧乾固した。これをMeOH 20ml、酢酸(AcOH) 20mlの混合液に溶解し、窒素気流下で攪拌した。攪拌を止め、10%パラジウム/カーボン(Pd/C) (51.60%含水物) 2.0gを加え、水素気流を通じた後、常温常圧で激しく攪拌して、反応を開始した。水素の吸収が終了したところで攪拌を止め、窒素ガスで水素ガスを置換した後、触媒を濾別した。母液を減圧濃縮したものに水を加え、もう一度減圧濃縮した。濃縮物をセファデックス G-10カラム(内径2.6cm×長さ60cm)で分離精製した。水で溶出し、1フラクションあたり5分(約10ml)で分画した。各フラクションの純度をTLC (展開溶媒は、n-ブタノール:酢酸:ピリジン:水=4:1:1:2 (BAPW系)を用いた。)で確認し(Rf値 0.39)、目的物のスポットを単一に与える画分を集め、減圧乾固した。この乾固物を15mlの水に溶解し、凍結乾燥し、無色アモルファス状目的物 1.0g を得た。ここまでの通算収率は28%であった。元素分析 C<sub>19</sub>H<sub>25</sub>N<sub>3</sub>O<sub>4</sub> MW.359.43 計算値 C 63.49、H 7.01、N 11.69。分析値 C 59.41、H 7.42、N 10.66。

【0011】実施例 2 [Pro-Phe-ProのN-アセチル化体(Ac-PFP)の合成]

実施例1で得られたPro-Phe-Pro 1.2g (3.3ミリモル)を水10mlに溶解し、これに N-アセチルスクシンイミド(N-ASI) 1.5g (10ミリモル)を加え、1N 水酸化カリウム水溶液でpHを約7に調整後室温で終夜攪拌した。反応液を濃縮後、セファデックス G-10カラム(内径2.6cm×長さ60cm)で分離精製した。各フラクションをTLCで確認し、濃縮乾固するとTLCで単一スポット(展開溶媒BAPW系 Rf=0.56)を与える無色アモルファス状目的物 1.2g (収率90%)が得られた。元素分析 C<sub>21</sub>H<sub>27</sub>N<sub>3</sub>O<sub>5</sub> MW.401.46 計算値 C 62.83、H 6.78、N 10.47。分析値 C 59.69、H 6.50、N 10.97。

【0012】実施例 3 [Pro-Phe-ProのC-アミド化体(PFP NH<sub>2</sub>)の合成]

Boc-Phe 2.3g (8.8ミリモル)、Pro NH<sub>2</sub> 1.0g (8.8ミリモル)、HOBt 1.33g (9.8ミリモル)をDMF 30mlに溶解し、氷冷攪拌下、DMF 10mlに懸濁した WSCD HCl (MW115.24 36.46) 1.7g (8.8ミリモル)を滴下した。TEA 1.7ml加え、万能pH試験紙でpH7~8であることを確認した

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後、4℃で終夜反応させた。反応後、実施例1と同様に処理し、Boc-Phe-Pro NH<sub>2</sub>を無色油状物として得た。これに4N 塩酸/ジオキサン 16ml、チオアニソール 1.5mlを加え、氷冷下1時間攪拌した。実施例1と同様に処理し、得られた残渣にZ-Pro 2.0g (7.9ミリモル)、HOBt 1.1g (8.8ミリモル)を加え、DMF 20mlに溶解した。氷冷攪拌下、DMF 10mlに懸濁したWSCD HCl 1.7g (8.7ミリモル)を滴下した。TEA 1.5mlでpH7~8に調整した後、4℃で終夜反応させた。反応後、実施例1と同様の処理を行い、Z-Pro-Phe-Pro NH<sub>2</sub>の油状物を得た。実施例1と同様にセファデックス LH-20カラムで精製し、TLCでほぼ単一スポットを得、続いて水素接触還元法により脱保護反応後、セファデックス G-10カラムで精製して、Et<sub>2</sub>Oから結晶化させ、無色結晶 0.2g (BAPW系 Rf=0.48)を得た。ここまでの通算収率は39%であった。元素分析 C<sub>19</sub>H<sub>26</sub>N<sub>4</sub>O<sub>3</sub> MW.358.44 計算値 C 60.37、H 7.31、N 15.63。分析値 C 60.48、H 7.38、N 14.55。

【0013】実施例 4 [Pro-Phe-ProのN-アセチル化、C-アミド化体(Ac-PFP NH<sub>2</sub>)の合成] 実施例3で得られたPFP NH<sub>2</sub> 1.0g (2.8ミリモル)に水6mlを加えて溶解し、実施例2と同様に反応・精製することによって目的物 0.9g (収率80%)が得られた。これはTLCで単一スポット(展開溶媒BAPW系 Rf=0.62)を与えた。元素分析 C<sub>21</sub>H<sub>28</sub>N<sub>4</sub>O<sub>4</sub> MW.400.25 計算値 C 62.98、H 7.05、N 13.99。分析値 C 58.91、H 6.68、N 13.01。

【0014】実施例 5 [Pro-Lys-Pro(PKP)の合成]

Boc-Lys(Cl-Z) t-Bu NH<sub>2</sub> 3.5g (7.1ミリモル)を400mlのEtOAcに溶解し、0.4Mクエン酸の10%NaCl水溶液、10%NaCl水溶液の順に洗浄した。EtOAc層に無水Na<sub>2</sub>SO<sub>4</sub>を加えて脱水後、溶媒を減圧留去し、アモルファス状の Boc-Lys(Cl-Z)を得た。これにProOBzl HCl 1.7g (7.1ミリモル)、HOBt 1.0g (7.7ミリモル)とWSCD HCl 1.5g (7.8ミリモル)を加え実施例1と同様に反応・精製し、Boc-Lys(Cl-Z)-ProOBzlを得た。得られた乾固物を実施例1と同様に酸処理してBoc基を除去し、得られた残渣にZ-Pro 1.7g (6.8ミリモル)、HOBt 1.0g (7.5ミリモル)、WSCD HCl 1.5g (7.8ミリモル)を加え実施例1と同様に反応・精製し、油状のZ-Pro-Lyz(Cl-Bzl)-ProOBzlを得た。更にこれをセファデックス LH-20カラム(内径2.6cm×長さ60cm)で精製し、TLCでほぼ単一のスポットを与えるフラクションを集め、減圧乾固した。これに少量のEtOAcに溶かしたEt<sub>2</sub>Oを加え、冷却することで結晶化した。結晶を濾取し、真空デシケーター中で減圧乾固し、無色結晶 2.68gを得た。この結晶をAcOH 36ml、MeOH 24mlに溶解し、実施例1と同様に水素接触還元法で全保護基の除去を行った。セファデックス G-10カラム(内径2.6cm×長さ60cm)で分離精製した。各フラクションをTLC (展開溶媒BAPW系 Rf=0.009)で確認し、目的のフラクションを集め、濃縮し、さらに凍結乾燥すると無色アモルファス状目的物 1.4gを得た。ここまでの通算



収率は63%であった。元素分析  $C_{16}H_{28}N_4O_4$  MW. 340.42 計算値 C 56.45, H 8.29, N 16.34. 分析値 C 54.06, H 8.59, N15.27.

【0015】実施例 6 [Pro-Lys-ProのN-アセチル化体(Ac-PKP)の合成]

実施例5で得られたPro-Lys-Proのアモルファス状物 1.2g(3.8ミリモル)を水10mlに溶解し、溶液をpH7に調整後、N-ASI 1.6g(11.3 ミリモル)を加え、室温で一晩攪拌した。反応液を濃縮乾固した後、残渣を少量のクロロホルム( $CHCl_3$ )に溶解し、 $CHCl_3$ :MeOH=10:1の溶液で作製したシリカゲル C-200カラム(内径2.2cm×長さ33cm)に層積後、 $CHCl_3$ :MeOH=10:1の溶液300mlで、そしてMeOHのみで溶出させ、約10mlのフラクションに分画し、各フラクションをTLCで確認して、目的物のスポットを与えるフラクション102から115までを集め、濃縮乾固した。これを少量の水に溶解後、凍結乾燥するとTLCで単一スポット(展開溶媒BAPW系 Rf=0.40)を与える無色アモルファス状物 1.25g(76%)が得られた。核磁気共鳴スペクトル(NMR)でシグナルの位置を満足した。 $^1H$  NMR(DMSO- $d_6$ ,  $\delta$ ) 1.82(3H, s) 2.15(3H, s).

【0016】実施例 7 [Pro-Lys-Pro NH<sub>2</sub>(PKP NH<sub>2</sub>)の合成]

Boc-Lys(Z) 3.5g(9.2ミリモル)、Pro NH<sub>2</sub> 1.1g(9.2ミリモル)、HOBt 1.34g(9.9ミリモル)、WSCD HCl 1.95g(10ミリモル)とを実施例1と同様に反応・精製し、目的物 Boc-Lys(Z)-Pro NH<sub>2</sub>を無色油状物として得た。実施例1と同様に処理して得られた残渣にZ-Pro 1.5g(6.1ミリモル)、HOBt 0.85g(6.3ミリモル)、WSCD HCl 1.3g(6.8ミリモル)とを加え、実施例1に記載した方法と同様に反応し、Z-Pro-Lys-Pro NH<sub>2</sub>を油状物として得、これを実施例1と同様にセファデックス LH-20カラムで精製してTLCでほぼ単一のスポットを与えるものを得た。続いて10%Pd/Cを用いた水素接触還元法により脱保護反応後、少量の水に溶解して、セファデックス G-10カラムで精製して凍結乾燥すると単一スポット(展開溶媒BAPW系 Rf=0.17)を与える目的物 1.1gが得られた。ここまでの通算収率は35%であった。 $C_{16}H_{29}N_5O_3$  MW 339.44 計算値 C 56.62, H 8.61, N 20.63. 分析値 C 51.56, H 8.83, N 18.63.

【0017】実施例 8 [Pro-Tyr-Pro(PYP)の合成]

Boc-Tyr(Bzl) 2.0g(5.3ミリモル)、ProOBzl HCl 1.3g(5.38ミリモル)、HOBt 0.81g(6.0ミリモル)、WSCD HCl 1.14g(5.9ミリモル)を実施例1と同様に反応させ、目的物 Boc-Tyr(Bzl)-ProOBzlを得た。この乾固物に実施例1と同様に酸処理してBoc基を除去し、得られた残渣に Z-Pro 1.3g(5.1ミリモル)、HOBt 0.76g(5.6ミリモル)、WSCD HCl 1.1g(5.6ミリモル)を滴下し、実施例1と同様に反応させた後、セファデックス LH-20カラム(内径2.6cm×長さ60cm)で分離精製した。減圧濃縮した後、これを少量のEtOAcに溶解し、Et<sub>2</sub>Oを加

え、冷却することで結晶化した。結晶を濾取し、減圧乾固して2.68gを得た。この結晶を水素接触還元法により脱保護したのち、セファデックス G-10 カラムで精製した。濃縮後、少量のMeOHに溶解し、Et<sub>2</sub>Oを添加して結晶化させ、冷却後結晶を濾集し、乾燥すると1.16gが得られた。ここまでの通算収率は55%であった。このものはTLCで単一スポット(展開溶媒BAPW系 Rf=0.32)を与えた。元素分析  $C_{19}H_{25}N_3O_5$  MW375.43 計算値 C 60.79, H 6.71, N 11.19. 分析値 C57.54, H 6.94, N 10.19.

【0018】実施例 9 [Pro-Tyr-ProのN-アセチル化体(Ac-PYP)の合成]

実施例8で得られたPro-Tyr-Pro 1g(2.6ミリモル)にN-ASI 1.1g(8ミリモル)を加え、実施例2と同様に反応させ、反応液をクロロホルム 20mlで抽出する操作を5回繰り返した。クロロホルム層をNa<sub>2</sub>SO<sub>4</sub>で脱水後減圧乾固した。これを少量の水に溶解し、凍結乾燥し、TLCで単一スポット(展開溶媒BAPW系 Rf=0.51)を与える無色アモルファス状物 0.68g(収率61%)を得た。元素分析  $C_{21}H_{27}N_3O_6$  MW417.46 計算値 C 60.42, H 6.52, N 10.07. 分析値 C 54.52, H 6.58, N 11.04.

【0019】実施例 10 [Pro-Tyr-Pro NH<sub>2</sub>(PYP NH<sub>2</sub>)の合成]

Boc-Tyr(Bzl) 3.25g(8.8ミリモル)、Pro NH<sub>2</sub> 1.0g(8.8ミリモル)、HOBt 1.2g(8.9ミリモル)にWSCD HCl 1.7g(9.0ミリモル)を加え、実施例1と同様に反応・精製し、Boc-Tyr(Bzl)-Pro NH<sub>2</sub>を無色油状物として得た。得られた乾固物を実施例1と同様に酸処理してBoc基を除去し、溶媒を減圧濃縮してEt<sub>2</sub>Oを加え結晶を析出させた。この結晶を濾取し、真空デシケーター中で減圧乾固した。乾固した結晶にZ-Pro 2.0g(7.9ミリモル)、HOBt 1.2g(8.9ミリモル)を加え、WSCD HCl 1.7g(9.0ミリモル)を滴下し、実施例1と同様に反応・精製し、油状物を得た。これを少量のメタノールに溶解し、セファデックス LH-20カラム(内径2.6cm×長さ60cm)にかけ、同溶媒で溶出した。目的のフラクションをTLCにて探し、1つに集めた後、減圧乾固し、アモルファス状目的物 4.9gを得た。これを水素接触還元法による脱保護反応を行なった後、セファデックス G-10カラムで精製後凍結乾燥し、TLCで単一スポット(展開溶媒BAPW系 Rf=0.42)を与える無色アモルファス状目的物 2.9gを得た。ここまでの通算収率は87%であった。元素分析  $C_{19}H_{26}N_4O_4$  MW 377.44計算値 C 60.95, H 7.60, N 14.96. 分析値 C 56.07, H 7.19, N 13.84.

【0020】実施例 11 [Pro-Tyr-ProのN-アセチル化、C-アミド化体(Ac-PYP NH<sub>2</sub>)の合成]

実施例10で得られたPYP NH<sub>2</sub> 1.0g(2.6ミリモル)にN-ASI 1.0g(7ミリモル)を加え、実施例2と同様に反応・精製して、TLCでほぼ単一スポット(展開溶媒BAPW系 Rf=0.61)を与えるフラクションを集め、濃縮し凍結

乾燥すると無色アモルファス状目的物 1.05g が得られた。ここまでの収率は96%であった。元素分析  $C_{21}H_{28}N_4O_6$  MW 416.48 計算値 C 60.56, H 6.78, N 13.45。分析値 C 55.11, H 6.01, N 13.09

【0021】実施例 12 [Tyr-Ser-Pro(YSP)の合成]

Boc-Ser(Bzl) 2.0g (6.8ミリモル)、ProOBzl HCl 1.6g (6.8ミリモル)、HOBt 1.0g (7.5ミリモル)をDMF 20mlに溶解し、氷冷下攪拌しながら、DMF 10mlに懸濁したWSCD HCl 1.4g (7.3ミリモル)に加え、実施例1に記載した方法と同様に反応させてBoc-Ser(Bzl)-ProOBzlを得た。実施例1に記載した方法と同様に酸処理による脱Boc基反応を行い、得られた残渣にZ-Tyr(Bzl) 2.6g (6.3ミリモル)、HOBt 0.85g (6.3ミリモル)を加え、WSCD HCl 1.3g (6.8ミリモル)を滴下し、実施例1と同様に反応させ、得られた残渣を少量のMeOHに溶かし、セファデックス LH-20カラムで精製した。目的のフラクションをTLCにて探し、集め減圧乾固した。これをEtOAc 50mlに溶解した後、250mlのEt<sub>2</sub>Oを加え、冷蔵庫に放置することで結晶化した。結晶を濾取し、減圧乾固した (2.40g)。この結晶を水素接触還元法による脱保護反応を行なった。セファデックス G-10カラムで精製し、凍結乾燥するとTLCで単一スポット (展開溶媒BAPW系 Rf=0.30) を与える無色アモルファス状目的物 1.70g が得られた。ここまでの通算収率は68%であった。元素分析  $C_{17}H_{23}N_3O_6$  MW 365.38 計算値 C 56.03, H 6.63, N 15.37。分析値 C 55.91, H 6.81, N 13.82。

【0022】実施例 13 [Tyr-Ser-ProのN-アセチル化体(Ac-YSP)の合成]

実施例12で得られたYSP 1.0g (YSP 2.7ミリモル)にN-ASI 1.1g (8ミリモル)を加え室温で2時間反応させた (pH約8)。反応液を濃縮後、セファデックス G-10カラムで精製し、TLCで単一のスポット (展開溶媒BAPW系 Rf=0.44) を与える目的物 0.6g が得られた。ここまでの通算収率は54%であった。元素分析  $C_{19}H_{25}N_3O_7$  MW 407.41 計算値 C 56.01, H 6.18, N 10.31。分析値 C 54.95, H 5.37, N 10.11。

【0023】実施例 14 [Tyr-Ser-Pro NH<sub>2</sub>(YSP NH<sub>2</sub>)の合成]

Boc-Ser(Bzl) 2.6g (8.8ミリモル)、Pro NH<sub>2</sub> 1.0g (8.8ミリモル)、HOBt 1.30g (9.7ミリモル)にWSCD HCl 2.0g (10ミリモル)を加え、実施例1と同様に反応させ、Boc-Ser(Bzl)-Pro NH<sub>2</sub>を油状物として得た。この油状物を実施例1に記載した方法と同様に酸処理し、溶媒を濃縮した後、Et<sub>2</sub>Oを加え、デカンテーションにて沈澱を得た。これを減圧乾固し、Z-Tyr(Bzl) 3.2g (7.9ミリモル)、HOBt 1.2g (8.7ミリモル)を加え、DMFに懸濁したWSCD HCl 1.7g (8.8ミリモル)を滴下した。溶媒を濃縮後、少量のEtOAcに溶解し、Et<sub>2</sub>Oを加え、冷却することで結晶化した。結晶を濾取し、減圧乾燥すると、

3.25g得られた。この結晶を水素接触還元法にて脱保護反応を行い、セファデックスG-10カラムで精製しTLCでは単一のスポット (展開溶媒BAPW系 Rf=0.44) を与える目的物のフラクションを集め濃縮し、凍結乾燥すると、無色アモルファス状物 1.7g が得られた。ここまでの通算収率は53%であった。元素分析  $C_{17}H_{24}N_4O_6$  MW 364.39 計算値 C 56.03, H 6.63, N 15.37。分析値 C 53.91, H 6.71, N 13.82。

【0024】実施例 15 [Tyr-Ser-ProのN-アセチル化、C-アミド化体(Ac-YSP NH<sub>2</sub>)の合成]

実施例14で得られたYSP NH<sub>2</sub> 0.8g (2.2ミリモル)に、N-ASI 1.0g (7ミリモル)を加え、実施例2に記載した方法と同様に反応させ処理し、乾固物を得た。しかしこれには、縮合剤由来物の混入が推定されたので、少量の水に溶解し、EtOAc 10mlで5回抽出を繰り返し水層を凍結乾燥すると無色アモルファス状物0.87g (収率 95%) が得られた。このものはTLCで単一スポット (展開溶媒BAPW系Rf=0.56) を与えた。

【0025】実施例 16 [Glu-Arg-Pro(ERP)の合成]

Boc-Arg(NO<sub>2</sub>) 1/2AcOEt 1/4H<sub>2</sub>O 3.7g (10ミリモル)、ProOBzl HCl 2.4g (10ミリモル)、HOBt 1.5g (11ミリモル)にWSCD HCl 2.1g (11.0ミリモル)を加え、実施例1と同様に反応させて処理し、Boc-Arg(NO<sub>2</sub>)-ProOBzlを油状物として得た。得られた油状物を実施例1と同様に酸処理した。得られた油状物にZ-Glu(Bzl)1.7g (5.0ミリモル)、HOBt 0.75g (5.7ミリモル)、WSCD HCl 1.1g (5.5ミリモル)を滴下し、実施例1と同様に反応した。得られた残渣を少量のEtOAcに溶解した後、Et<sub>2</sub>Oを加え、冷却すると結晶化した。結晶を濾取し、減圧乾固し2.3gの結晶を得た。この結晶を水素接触還元法で全保護基の除去反応を行ない、得られた残渣をセファデックス G-10カラムで精製して、TLCで単一のスポット (展開溶媒BAPW系 Rf=0.007) を与えるフラクションを集め、濃縮し、凍結乾燥して無色アモルファス状目的物 1.3gを得た。ここまでの通算収率は32%であった。元素分析  $C_{16}H_{28}N_6O_6$  MW 400.44 計算値 C 47.99, H 7.05, N 20.99。分析値 C 45.84, H 7.55, N 19.52。

【0026】実施例 17 [Glu-Arg-ProのN-アセチル化体(Ac-ERP)の合成]

実施例16で得られた ERP 1.1g (2.7ミリモル)に、N-ASI 1.1g (7.1ミリモル)を加え、一晚室温で攪拌した。反応液を濃縮後、セファデックス G-10 カラムで精製し、各フラクションを集め濃縮し、凍結乾燥して無色アモルファス状目的物 0.80g が得られた。ここまでの通算収率は67%であった。このものはTLCで単一スポット (展開溶媒BAPW系 Rf=0.16) を与えた。元素分析  $C_{18}H_{30}N_6O_7$  MW 442.47 計算値 C 48.86, H 6.83, N 18.99。分析値 C 45.89, H 7.03, N 18.07。

【0027】実施例 18 [Glu-Arg-ProのC-アミド化

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体(ERP NH<sub>2</sub>)の合成]

Boc-Arg(NO<sub>2</sub>) 1/2AcOEt 1/4H<sub>2</sub>O 3.7g (10.0ミリモル)、ProOBzl HCl 2.4g (10ミリモル)、HOBt 1.49g (11ミリモル)にWCD HCl 2.1g (11ミリモル)を加え、実施例1と同様に反応し、EtOAcの代わりにクロロホルムを用いて処理した。クロロホルム層を減圧濃縮した残渣に、EtOAcを加えると白濁した。さらにEt<sub>2</sub>Oを加えて4℃で一晩放置して得られたガム状沈澱物をデカンテーションして得、減圧乾固した。得られた乾固物を実施例1と同様に酸処理してBoc基を除去し、反応中生じた沈澱物をデカンテーションで得、減圧乾固した。これにZ-Glu(Bzl) 1.7g (5.0ミリモル)、HOBt 0.75g (5.5ミリモル)を加えWCD HCl 1.1g (5.7ミリモル)を滴下し、実施例1と同様に反応し、処理をした後、カラムで精製して無色油状物を得た。この油状物を水素接触還元法で全保護基の除去反応を行なった後、セファデックス G-10カラムで精製して、TLCでほぼ単一スポット (展開溶媒BAPW系 Rf=0.21) のフラクションを集め結晶性残渣を得た。これに冷MeOHを加えて結晶をほぐし、濾集すると0.2gが得られた。ここまでの通算収率は6%であった。元素分析C<sub>16</sub>H<sub>29</sub>N<sub>5</sub>O<sub>6</sub> MW399.45 計算値 C 48.11、H 7.32、N 24.55。分析値 C 46.61、H 6.92、N 22.34。

【0028】実施例 19 [Pro-Thr-Trp(PTW)の合成]

Boc-ThrOSu 3.7g (12ミリモル)、TrpOBzl HCl 3.8g (12ミリモル)のDMF溶液に、氷冷攪拌下、TEAを加えて、pH7~8であることを確認後、一晩反応させた。実施例1と同様に後処理して、Boc-Thr-TrpOBzlを油状物として得た。続いて実施例1と同様に酸処理して、Boc基を除去し得られた残渣にZ-Pro 2.8g (11ミリモル)を加え、DCCD 2.5g (12ミリモル)のDMF 10ml溶液を滴下し、4℃で一晩反応させた。沈澱物を濾別後、濾液を減圧濃縮し、実施例1と同様に処理してアモルファス状物 4.21g が得られた。このうち2gを水素接触還元法で保護基を除去し、得られた残渣を少量の水に溶解し、セファデックス G-10カラムで精製して、TLCでほぼ単一スポット (展開溶媒BAPW系 Rf=0.33) を与えるフラクションを集め減圧乾固した。残渣に少量のMeOHを加えて、結晶化させ、冷却後、結晶を濾取し乾燥し1.40gを得た。ここまでの通算収率は30%であった。元素分析 C<sub>20</sub>H<sub>25</sub>N<sub>4</sub>O<sub>6</sub> MW 402.22 計算値 C 59.69、H 6.51、N 13.91。分析値 C 55.90、H 6.41、N 12.75。

【0029】実施例 20 [Pro-Thr-Trp NH<sub>2</sub>(PTW NH<sub>2</sub>)の合成]

Boc-ThrOSu 6.0g (19ミリモル)をDMF 50mlに溶解し、Trp NH<sub>2</sub>塩酸塩 4.6g (19ミリモル)とHOBt 2.9g (21ミリモル)とを粉末のまま加え攪拌溶解した。氷冷攪拌下にTEAを添加し、pH7~8であることを確認後WCD HCl 4.1g (21ミリモル)のDMF 10ml溶液を加え、4℃で一晩反応させた。沈澱物を濾去し、濾液を減圧濃縮後 EtOAc 500

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mlを加えて、実施例1と同様に処理することによって目的物Boc-Thr-Trp NH<sub>2</sub>を無色油状物として8.3g(87%)を得た。これを実施例1と同様に反応させ処理して油状物 7.2g (16ミリモル, 98%)を得た。続いてこれに Z-Pro 4.1g (16ミリモル)、HOBt 2.5g(18ミリモル)、WCD HCl 3.5g (18ミリモル)を加え、実施例1と同様に反応させ処理して、Z-Pro-Thr-Trp NH<sub>2</sub>をアモルファス状物として7.3g(70%)を得た。このアモルファス状物 2.5gに10% Pd/C 1gを加えて実施例1と同様に反応・処理後、脱保護反応を行った。得られたものを少量の水に溶解してセファデックス G-10カラムで精製し、凍結乾燥すると、単一スポット (展開溶媒BAPW系 Rf=0.47) からなる目的物 1.0gが得られた。元素分析 C<sub>20</sub>H<sub>27</sub>N<sub>5</sub>O<sub>4</sub> MW.401.33 計算値 C 59.80、H 6.78、N 17.46。分析値 C 57.63、H 6.94、N 16.27。

【0030】実施例 21 [Pro-Thr-Trp NH<sub>2</sub>のN-アセチル化体(Ac-PTW NH<sub>2</sub>)の合成]

実施例20で得られたPTW NH<sub>2</sub> 0.95g (2.4ミリモル)にN-ASI 0.9g (6ミリモル)を加え、室温で一晩反応させたのち、減圧下に濃縮乾固した。少量のクロロホルムに溶かし、クロロホルム:メタノール=30:1の混液で作製したシリカゲル C-200 (和光純薬製)カラム(内径2.6cm×長さ45cm)に層積し、クロロホルム:メタノール=30:1混液 400ml、10:1混液 300ml、メタノールのみ500mlで溶出させ、各フラクション(約10ml)をTLCでチェック後、フラクション72から75までを集めた。濃縮乾固後、残渣を少量の水に溶解し凍結乾燥すると無色アモルファス状物 0.8gが得られた。ここまでの通算収率は75%であった。このものはTLCで単一スポット (展開溶媒BAPW系 Rf=0.64)を与えた。元素分析 C<sub>22</sub>H<sub>29</sub>N<sub>5</sub>O<sub>5</sub> MW443.50 計算値 C 59.58、H 6.59、N 15.79。分析値 C 56.98、H 6.54、N 15.34。3gが得られた。

【0031】実施例 22 [thioPro-Thr-Trp(P'TW)の合成]

Boc-ThrOSu 3.7g (12ミリモル)、TrpOBzl HCl 3.8g (12ミリモル)のDMF溶液に、氷冷攪拌下、TEAを加えて、pH7~8であることを確認後、一晩反応させた。実施例1と同様に後処理して、Boc-Thr-TrpOBzlを油状物として得た。続いて実施例1と同様に酸処理してBoc基を除去し、得られた残渣にBoc-thioPro2.8g (12ミリモル)を加え、HOBt 1.784g (13.2ミリモル)、WCD HCl 2.53g (13.2ミリモル)のDMF 10ml溶液を滴下し、4℃で一晩反応させた。沈澱物を濾別後、濾液を減圧濃縮し、実施例1と同様に処理してアモルファス状物 6.3gが得られた。カップリングにより得られたBoc-thioPro-Thr-TrpOBzl 6.3gのHFによる保護基の切断は以下の通りに行った。Boc-thioPro-Thr-TrpOBzl 6.3gを10等分して、それぞれHFにより切断した。即ち、Boc-thioPro-Thr-TrpOBzlを反応容器に採り、チオアニソール 11mlを加えて室温に1時間置いた。反応容器をドライアイス-アセトン浴

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で冷却下、HF 100mlを導入、0℃で1時間攪拌しながら反応させた。水流アスピレーターでHFを減圧除去し、更に真空ポンプにて乾燥させた。得られた残渣を10%酢酸水溶液100mlにて溶解し、50mlのジエチルエーテルで2回洗浄し、残存するチオアニソールを除去した。酢酸水溶液画分を中和して、濃縮乾固させたもの3.2gを少量のクロロホルム-メタノール混液(5:1)に溶解し、シリカゲルカラムクロマトグラフィーにより精製し、目的物を得た。収量0.6g。TLC (BAPW系Rf=0.62)は単一のスポットを与えた。元素分析 C<sub>19</sub>H<sub>25</sub>N<sub>4</sub>O<sub>6</sub>S MW420.53 計算値 C 54.22, H 5.99, N 13.35。分析値 C 55.90, H 6.41, N 12.75。

【0032】実施例 23 [D-Pro-Thr-Trp NH<sub>2</sub> (D-PTW NH<sub>2</sub>: ProはD体)の合成]  
Boc-ThrOSu 3.7g (12ミリモル)、TrpOBzl HCl 3.8g (12ミリモル)のDMF溶液に、氷冷攪拌下、TEAを加えて、pH7-8であることを確認後、一晚反応させた。実施例1と同様に後処理して、Boc-Thr-TrpOBzlを油状物として得た。続いて実施例1と同様に酸処理して、Boc基を除去し、得られた残渣にZ-D-Pro 2.8g (11ミリモル)を加え、DCCD 2.5g (12ミリモル)のDMF 10ml溶液を滴下し、4℃で一晩反応させた。沈澱物を分別後、母液を減圧濃縮し、実施例1と同様に処理してアモルファス状物4.21gが得られた。このうち2gを接触還元法で保護基を除去し、得られた残さを少量の水に溶解し、セファデックスG-10カラムで精製して、TLCでほぼ単一スポットを与えるフラクションを集め減圧乾固した。残渣に少量のMeOHを加えて、結晶化させ、冷却後、結晶を濾取し\*

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\*乾燥し1.40g (通算収率30%)を得た (BAPW系 Rf=0.33)。元素分析 C<sub>20</sub>H<sub>25</sub>N<sub>4</sub>O<sub>6</sub> MW 402.22 計算値 C 59.69, H 6.51, N 13.91。分析値 C 55.90, H 6.41, N 12.75。

【0033】実施例 24 [Glu-D-Arg (E-D-R: ArgはD体)の合成]

Z-Glu(Bzl)OH 3.4g (10ミリモル)、D-Arg(NO<sub>2</sub>)OBzl Tos 4.82g (10ミリモル)、HOBt 1.5g (11ミリモル)をDMF 100mlに溶解、氷冷下、WSCD HCl 2.1g (11ミリモル)を加え、実施例1と同様に反応させ、後処理を行って、Z-Glu(Bzl)-D-Arg(NO<sub>2</sub>)OBzlの油状物5.3g (8ミリモル)を得た。この油状物を接触還元法にて脱保護反応を行い、セファデックスG-10カラムで精製し、TLCでほぼ単一のスポットを与える目的物のフラクションを集め、濃縮、凍結乾燥を行った。無色アモルファス状物として1.82g (6ミリモル)を得た (TLC: BAPW系 Rf=0.58)。元素分析 C<sub>11</sub>H<sub>21</sub>N<sub>5</sub>O<sub>6</sub> MW 303.33 計算値 C 43.52, H 6.92, N 23.14。分析値 C 42.98, H 6.88, N 22.76

【0034】試験例 1 [フォーカス形成阻害試験]  
8穴ラブリックチャンバー (スライドガラスサイズ) に癌原遺伝子erbB-2で形質転換したNIH3T3細胞を入れ、1穴をコントロールとして残し、7穴にサンプルを添加し、37℃の5%炭酸ガスインキュベーターで培養し、24時間毎に5日間光学顕微鏡で細胞増殖状態 (フォーカス数) を観察し、毎回写真撮影した (サンプル最終濃度 250μg/ml) その結果の一部を表1 (表1) に示す。

【0035】

【表1】

表1 フォーカスアッセイ

サンプル (実施例番号) \ 日数	フォーカス数				
	1	2	3	4	5
9	0	0	0	2	6
13	0	1	1	4	5
17	0	0	0	3	17

【0036】試験例 2 [MTT測定法]

10%牛血清を含むDMF培地に種々の癌遺伝子で癌化したNIH3T3細胞を5×10<sup>4</sup>細胞/mlとなるように懸濁しその100μlを96穴平底プレートの各ウェルに分注した。このうち3ウェルには同量の培養液のみを加え、陰性コントロールとした。このプレートを37℃、5%炭酸ガスインキュベーター中で一晩培養後880μg/mlの濃度に水や例えばアセトン、ジメチルホルムアミド、ジメチルスルホキシド、アルコールなどの有機溶媒で調製した本発明のトリペプチド及びその誘導体溶液10μlを各ウェルに添加した (終濃度80μg/ml)。これを37℃、5%炭酸ガスインキュベーター

40※ベーター中で2時間培養後5mg/ml濃度のMTT、PBS(-)溶液10μlを全ウェルに添加し37℃、5%炭酸ガスインキュベーター内で2時間培養した。プレートホルダーを装着した冷却低速遠心機を用いて各プレートを3000rpm、15分間遠心した。プレートを遠心機から取り出し、各ウェルの上清を吸引して取り除き、150μlのDMSOを添加してミキサーで攪拌後、イムノリーダーにかけて540nmにおける吸光度を測定した (比色定量)。その結果の一部を表2 (表2) に示す。表2中F25、PH1、S59、erbB-2、v-src、v-mos、SV-40はそれぞれc-H-ras癌原遺伝子、v-H-ras癌原遺伝子、K-H-ras癌原遺伝子、erbB-2癌原遺伝子

子、v-src癌原遺伝子、v-mos癌原遺伝子、SV-40癌原遺伝子で癌化したNIH3T3細胞であることを示す。 \* 【0037】  
\* 【表2】

表2 MTT法による in vitro 制癌効果試験結果

実施例番号、細胞	F25	PH1	S59	erbB2	v-src	SV40	v-mos	生存率	
								++	0-50%
1	+	+	±	+	+	+	++	+	50-100%
2	±	+	±	±	+	+	+	±	100-120%
3	+	+	+	++	±	+	++		
4	++	++	++	++	++	++	++		
5	±	±	±	+	+	±	±		
6	+	±	±	+	+	±	+		
7	±	+	±	+	+	±	+		
8	+	+	+	+	+	+	+		
9	+	+	+	++	+	±	+		
10	±	+		+	+				
11	++	+	+	++	+	++	++		
12	+	±	+	+	+	+	+		
13	+	+	±	+	+	+	+		
14	±	+	+	+	+	±	±		
15	+	+	+	+	+	±	++		
16	±	+		+	+				
17	+	±	+	±	+	+	++		
18	+	+		±	+				
19	+	+	+	++	++	+	+		
20	+	+	+	+	+	+	+		
21	+	+	+	+	+	+	+		
22	++	++	++	++	++	++	++		
23	±	+	+	+	±	+	+		
24	++	++	++	++	++	++	++		

## 【0038】

【発明の効果】本発明によりトリペプチドを含有する全く新規な医薬組成物が提供される。本発明の医薬組成物はその有効成分がペプチドであり、その物は生体内でア※

※ミノ酸に分解され代謝される。そのため、本発明の医薬組成物は生体に投与した場合に副作用を起こす危険性が極めて少なく、医薬品として有効である。

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CLAIMS

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[Claim(s)]

[Claim 1] The tripeptide shown by Pro-A-B (however, A=Phe, Lys, Asn and Tyr, Thr; B=Pro, and Trp are shown.), or the remedy constituent which contains those salts accepted physiologically as an active principle.

[Claim 2] The tripeptide shown by C-D-Pro (however, C=Tyr, Glu, Pro; D=Asn, and Ser, Arg and Tyr are shown), or the remedy constituent which contains those salts accepted physiologically as an active principle.

[Claim 3] The tripeptide shown by Thioproline-Thr-Trp, or the remedy constituent which contains those salts accepted physiologically as an active principle.

[Claim 4] The remedy constituent which contains the dipeptides shown by Glu-Arg, or those salts that were accepted physiologically as an active principle.

[Claim 5] A remedy constituent given in any 1 term of the 1st term of a patent claim characterized by being the derivatives by which the amino terminal of tripeptide was acylated, or those salts that were accepted physiologically - 4 term.

[Claim 6] A remedy constituent given in any 1 term of the 1st term of a patent claim characterized by being the derivatives with which the C terminal of tripeptide was amidated, or those salts that were accepted physiologically - 4 term.

[Claim 7] A remedy constituent given in the 1-6th terms of a patent claim which are an anticancer agent.

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DETAILED DESCRIPTION

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[Detailed Description of the Invention]

[0001]

[Field of the Invention] This invention relates to the remedy constituent containing tripeptide or a dipeptide useful as an anticancer agent.

[0002]

[Description of the Prior Art] Research of the inhibitor of thrombin activity is known about using the tripeptide and its derivative as drugs (journal medical chemistry: vol.37, p.2122, 1994 year \*\*:vol.36, p. 1993 [ 300 or ]). Moreover, the development research as drugs of a comparatively short peptide and its derivative is seen by for example, a \*\*\*\*\* No. 502154 [ four to ] official report, a \*\*\*\*\* No. 502306 [ four to ] official report, a \*\*\*\*\* No. 502308 [ four to ] official report, a \*\*\*\*\* No. 502309 [ four to ] official report, etc. In these researches, a prolyl radical is contained in the amino acid sequence of the peptide, and the number of amino acid of the die length of an array is five or more. These peptides can also be said to be the TNF amelioration peptide guided from the tumor necrosis factor (TNF). Thus, some researches which use a short peptide as drugs were made. that intracellular is easy to be incorporated considers a short peptide enough -- having -- in addition -- and since it is decomposed in the living body and they serve as harmless amino acid, it is thought that the side effect over administration to a living body is hardly produced. Therefore, it is thought that these short peptides begin cancer and can serve as promising drugs as a remedy of other diseases in the future. However, about the utilization as an anticancer agent of a short peptide, it was not known at all until now.

[0003]

[Problem(s) to be Solved by the Invention] While this invention persons investigated the growth mechanism of a cancer cell, they came to get to know that the protein-protein interaction has determined the important metabolic fate. Although it was generally thought that the complicated structure of the protein itself was participating in association with protein and protein, the result of detailed research of the joint domain of a protease and its inhibitor showed that the peptide chain with a very short part of the protein of big molecular weight was participating in proteinic association. When making the peptide equivalent to the joint domain of the protein which participates in growth of a cancer cell from the above thing live together in intracellular, association with this protein and other protein could be prevented, and since it was possible to control the function of this protein as a result, it was thought that such a short peptide was useful as an anticancer agent. The object of this invention is to offer the short peptide which has the carcinostatic effectiveness, and the object of this invention still more specifically has it in offering the remedy constituent containing the tripeptide which has a specific amino acid sequence, dipeptides, or those derivatives, and it is for other objects of this invention to offer the remedy constituent containing the salt accepted physiologically [ the tripeptide which has a specific amino acid sequence, a dipeptide, or its derivative ].

[0004]

[Means for Solving the Problem] The various oncogene products as an amino acid sequence of tripeptide with the promising this invention persons, For example K-Sam, Yes, Ret, Kit and Fms, ErbB,



Met, Ros, Sea, Trk, Src, Fgr, Fyn, Lyn, The consensus sequence of the oncogene product [ Arg / Lck, Hck, Abl, ] which has the array further called the Sark homology (SH) domain was searched, and the array which participates in proteinic association through research of the tripeptide equivalent to a joint domain etc. was predicted. Consequently, it finds out having the effectiveness that the tripeptide which has a fixed amino acid sequence checks growth of a cancer cell, and came to complete this invention based on this knowledge.

[0005] Namely, the tripeptide this invention is indicated to be by Pro-A-B (however, A=Phe, Lys, Asn and Tyr, Thr; B=Pro, and Trp are shown.), Or the remedy constituent which contains those salts accepted physiologically as an active principle, The tripeptide shown by Thioproline-Thr-Trp (Following thioPro or P'), Or the remedy constituent which contains those salts accepted physiologically as an active principle, The dipeptide shown by Glu-Arg, or the remedy constituent which contains those salts accepted physiologically as an active principle, The remedy constituent which contains the tripeptide shown by C-D-Pro (however, C=Tyr, Glu, Pro; D=Asn, and Ser, Arg and Tyr are shown) or those salts that were accepted physiologically as an active principle is offered.

[0006]

[Embodiment of the Invention] As an amino acid sequence of the tripeptide concerning this invention, they are Pro-Phe-Pro, Pro-Lys-Pro, Pro-Tyr-Pro, Tyr-Ser-Pro, Glu-Arg-Pro, Pro-Tyr-Trp, etc. As an amino acid sequence of the dipeptide concerning this invention, it is Glu-Arg etc. In this invention, that by which the amino acid of the amino terminal of the above-mentioned tripeptide and a dipeptide was acylated, the thing by which the amino acid of a C terminal was amidated, and the thing with which ends were embellished like the above are also used. As an N-acylation derivative of the tripeptide of this invention, and a dipeptide, it is a formyl group, an acetyl group, an aryl carbonyl group, and an aromatic series carbonyl group derivative, and the amino group, an alkylamino radical, and an aromatic series amino-group derivative can be raised as a C-amidation derivative. As a salt accepted physiologically [ the tripeptide of this invention, a dipeptide, and its derivative ], a hydrochloric acid, a citric acid, a phosphoric acid, a tartaric acid, a lactic acid, an acetic acid, a formic acid, a fumaric acid, a maleic acid, a succinic acid, etc. are raised.

[0007] Composition of the tripeptide concerning this invention, a dipeptide, and its derivative is the Japanese Biochemical Society edit \*\*\*\*\* experiment lecture 2. As proteinic chemistry (below) has a publication, it is easily compoundable by any approach of a solid phase technique or a liquid phase process. Purification of the tripeptide concerning this invention, a dipeptide, and its derivative can be performed by the usual approaches, such as a silica gel column chromatography and an ion-exchange column chromatography. There is no definition also in an eluate and it can use the usual organic solvents, such as water, a methanol, and chloroform, for it. It is 1-ethyl about the amino acid of the commercial item which protected the carboxyl group by benzyl (Bzl radical) first when describing composition of the tripeptide concerning this invention, a dipeptide, and its derivative in more detail. - 3-(3-dimethylaminopropyl)- It condenses using condensing agents, such as a carbodiimide hydrochloride (WSCD HCl) and dicyclohexylcarbodiimide (DCCD), and a dipeptide is obtained. As a solvent used for the condensation reaction of the tripeptide of this invention, and a dipeptide, N,N-dimethylformamide (DMF), dimethyl sulfoxide (DMSO), an acetonitrile, 1,4-dioxane, tetrahydro furans (THF), and such mixture are suitable. Clearance of the protective group of tripeptide and a dipeptide can use the hydrogen catalytic reduction method or liquefaction hydrogen fluoride (HF) which made the catalyst a trifluoro methansulfonic acid method (TMSFA law), and palladium/carbon. Generally as a solvent used with a hydrogen catalytic reduction method, it is used, and is satisfactory. A methanol (it may outline Following MeOH), acetic acids, or such mixture are suitable. The general approach of thin-layer chromatography (TLC) can be used for the reaction progress in each synthetic step, or the check of purity. In an expansion solvent, the check of a spot can use the hydrogen bromide (HBr)-ninhydrin method using a chloroform-methanol system and an n-butanol-acetic-acid-pyridine-water (4:1:1:2) system.

[0008] Although there is especially no constraint except the remedy constituent of this invention containing the tripeptide obtained as mentioned above, a dipeptide and its derivative, or those salts that

were accepted physiologically as an active principle, the additive usually used on the occasion of pharmaceutical-preparation-izing of a compound may also be included. The remedy constituent of this invention may be used as an anticancer agent which has the outstanding activity. Adapted \*\*\*\* are leukemia, an osteosarcoma, a breast cancer, an ovarian cancer, gastric cancer, colon cancer, lung cancer, etc. Although the medication method of the remedy constituent of this invention naturally changes with symptoms for administration, it divides and medicates 1 time or several times with the adult 1 person 1 Japanese hits 0.01-10g. In addition, the side effect of the remedy constituent of this invention is satisfactory in any way in anticipated-use extent.

[0009] The judgment of the carcinostatic effectiveness can add the tripeptide and its derivative, or these salts that were accepted physiologically of this invention to the culture medium of the culture cell strain which cancerated by various oncogenes, and can observe change of the gestalt of a cell, a reproductive rate, a survival rate, etc. by cultivating under the usual condition. For example, it is convenient if you use 3-[4 and 5-dimethyl thiazole-2-IRU]-2 and 5 diphenyl-tetrazolium bromine salt method (the MTT method) for reproductive rate depressor effect measurement of a cancer cell. Culture cell strains which cancerated as a cancer cell stock by oncogenes, such as v-Ha-ras, Ki-ras, v-src, c-erbB -2, v-mos, and SV40, such as NIH3 T3 fibroblast and 3Y1 rat fibroblast, can be fried. This invention is not limited by this although an example explains this invention concretely below.

[0010]

[Example]

Example 1 [Composition of Pro-Phe-Pro (PFP)]

Boc-Phe 2.7g (10 millimol), ProOBzl HCl 2.4g (10 millimol), and 1-hydroxy benzotriazol (HOBt) 1.5g (11 millimol) were dissolved in DMF 30ml, and WSCD HCl 1.7g (11 millimol) suspended in DMF 10ml was dropped at the bottom of ice-cooling stirring. triethylamine (TEA) -- after adding 1.8ml and checking that it is pH 7-8 with the omnipotent pH indicator paper, it stirred at the room temperature all night. After filtering and removing the precipitation which deposited and carrying out vacuum concentration of the filtrate, 300 ml ethyl acetate (EtOAc) was added and it washed in order of 10% salt (NaCl) water solution of 4% sodium hydrogencarbonate (NaHCO<sub>3</sub>), 10%NaCl water solution, 10% NaCl water solution of 0.4M citric acid, and 10%NaCl water solution. Anhydrous sodium sulfate (Na<sub>2</sub>SO<sub>4</sub>) was added to the EtOAc layer, reduced pressure distilling off of the solvent was carried out after dehydration, and reduced pressure hardening by drying of the residue was carried out in the desiccator. It is 4 Ns to this hardening-by-drying object. A hydrochloric acid/dioxane 40ml, thioanisole 4ml was added and it reacted under ice-cooling for 2 hours. Dioxane after carrying out vacuum concentration of this The actuation which adds and condenses 50ml was repeated twice, and the actuation which adds and condenses diethylether (Et<sub>2</sub>O) similarly was repeated twice. Z-Pro 1.5g (6.2 millimol) and HOBt 0.84g (6.8 millimol) were added to the hardening-by-drying object which carried out reduced pressure hardening by drying of this, and it dissolved in it at DMF 30ml. WSCD HCl 1.2g (6.8 millimol) suspended in DMF 10ml was dropped under ice-cooling stirring. After preparing to pH 7-8 by TEA 1.6ml, it was made to react at a room temperature for 1.5 hours. After making it react at 4 more degrees C all night, the precipitation which deposited was filtered and removed and vacuum concentration of the filtrate was carried out. EtOAc 300ml was added and it washed in order of 10% NaCl water solution of 4%NaHCO<sub>3</sub>, 10%NaCl water solution, 10%NaCl water solution of 0.4M citric acid, and 10%NaCl water solution. Anhydrous Na<sub>2</sub>SO<sub>4</sub> was added to the EtOAc layer, reduced pressure distilling off of the solvent was carried out after dehydration, and reduced pressure hardening by drying of the residue was carried out in the desiccator. A hardening-by-drying object is dissolved in a small amount of methanol (MeOH), and it is sephadex. Separation purification was carried out in LH-20 column (bore [ of 3cm ] x die length of 55cm). It was eluted with this solvent and fractionation was carried out in 5 minutes (about 8ml) per one fraction. By TLC (the system of chloroform-MeOH was used for the Kieselgel 60F 254 expansion solvent.), after the check, the fractions containing the specified substance were collected and reduced pressure hardening by drying was carried out about each fraction. This was dissolved in the mixed liquor of MeOH 20ml and a 20 ml acetic acid (AcOH), and it stirred under the nitrogen air current. It is stirring A stop and 10% After adding palladium / carbon (Pd/C)

(51.60% hydrated compound) 2.0g and leading a hydrogen air current, it stirred violently by ordinary temperature ordinary pressure, and the reaction was started. After it permuted the stop in the place which absorption of hydrogen ended and nitrogen gas permuted hydrogen gas for stirring, the catalyst was carried out the \*\* exception. Water was added to what carried out vacuum concentration of the filtrate, and vacuum concentration was carried out once again. It is sephadex about a concentrate. Separation purification was carried out in G-10 column (bore [ of 2.6cm ] x die length of 60cm). It was eluted with water and fractionation was carried out in 5 minutes (about 10ml) per one fraction. The purity of each fraction was checked by TLC (n-butanol:acetic-acid:pyridine:water =4:1:1:2 (BAPW system) was used for the expansion solvent.) (Rf value 0.39), the fractions which give the spot of the specified substance individually were collected, and reduced pressure hardening by drying was carried out. This hardening-by-drying object is dissolved in 15ml water, and it freeze-dries, and is the colorless amorphous-like specified substance. 1.0g It obtained. The overall yield so far was 28%. Elemental analysis C<sub>19</sub>H<sub>25</sub>N<sub>3</sub>O<sub>4</sub> MW.359.43 Calculated value C 63.49, H 7.01, N 11.69. Analysis value C 59.41, H 7.42, N 10.66.

[0011] Example 2 [Composition of N-acetylation object (Ac-PFP) of Pro-Phe-Pro]

Pro-Phe-Pro 1.2g (3.3 millimol) obtained in the example 1 is dissolved in 10ml of water, and it is this. N-acetyl succinimide (N-ASI) 1.5g (10 millimol) is added, and it is 1 N. pH was stirred at the room temperature after preparation to about 7 with the potassium-hydroxide water solution all night. Sephadex after condensing reaction mixture Separation purification was carried out in G-10 column (bore [ of 2.6cm ] x die length of 60cm). The colorless amorphous-like specified substance which will give a single spot (expansion solvent BAPW system Rf=0.56) by TLC if each fraction is checked by TLC and concentration hardening by drying is carried out 1.2g (90% of yield) was obtained. Elemental analysis C<sub>21</sub>H<sub>27</sub>N<sub>3</sub>O<sub>5</sub> MW.401.46 Calculated value C 62.83, H 6.78, N 10.47. Analysis value C 59.69, H 6.50, N 10.97.

[0012] Example 3 [Composition of C-amidation object (PFP NH<sub>2</sub>) of Pro-Phe-Pro]

Boc-Phe 2.3g (8.8 millimol), Pro NH<sub>2</sub> 1.0g (8.8 millimol), and HOBt 1.33g (9.8 millimol) were dissolved in DMF 30ml, and it suspended in DMF 10ml under ice-cooling stirring. WSCD HCl (MW115.24 36.46) 1.7g (8.8 millimol) was dropped. After checking that it is pH 7-8 with the omnipotent pH indicator paper [ TEA 1.7ml ], it was made to react at 4 degrees C all night. It processed like the example 1 after the reaction, and Boc-Phe-Pro NH<sub>2</sub> was obtained as colorless oily matter. It is 4 Ns to this. A hydrochloric acid/dioxane 16ml, thioanisole 1.5ml was added and it stirred under ice-cooling for 1 hour. It processed like the example 1, Z-Pro 2.0g (7.9 millimol) and HOBt 1.1g (8.8 millimol) were added to the obtained residue, and it dissolved in DMF 20ml. WSCD HCl 1.7g (8.7 millimol) suspended in DMF 10ml was dropped under ice-cooling stirring. After preparing to pH 7-8 by TEA 1.5ml, it was made to react at 4 degrees C all night. The same processing as an example 1 was performed after the reaction, and the oily matter of Z-Pro-Phe-Pro NH<sub>2</sub> was obtained. It is sephadex like an example 1. LH-20 column refines, and by TLC, a single spot is obtained mostly, it continues, and they are after a deprotection reaction and sephadex by the hydrogen catalytic reduction method. G-10 column refines, and it is made to crystallize from Et<sub>2</sub>O, and is a colorless crystal. 0.2g (BAPW system Rf=0.48) was obtained. The overall yield so far was 39%. Elemental analysis C<sub>19</sub>H<sub>26</sub>N<sub>4</sub>O<sub>3</sub> MW.358.44 Calculated value C 60.37, H 7.31, N 15.63. Analysis value C 60.48, H 7.38, N 14.55.

[0013] Example 4 [Composition of N-acetylation of Pro-Phe-Pro, and C-amidation object (Ac-PFP NH<sub>2</sub>)] It is the specified substance by adding 6ml of water, dissolving in PFP NH<sub>2</sub> 1.0g (2.8 millimol) obtained in the example 3, and reacting and refining like an example 2. 0.9g (80% of yield) was obtained. This gave the single spot (expansion solvent BAPW system Rf=0.62) by TLC. Elemental analysis C<sub>21</sub>H<sub>28</sub>N<sub>4</sub>O<sub>4</sub> MW.400.25 Calculated value C 62.98, H 7.05, N 13.99. Analysis value C 58.91, H 6.68, N 13.01.

[0014] Example 5 [Composition of Pro-Lys-Pro (PKP)]

It dissolved in 400ml EtOAc and Boc-Lys (Cl-Z) t-Bu NH<sub>2</sub> 3.5g (7.1 millimol) was washed in order of 10%NaCl water solution of 0.4M citric acid, and 10%NaCl water solution. Anhydrous Na<sub>2</sub>SO<sub>4</sub> is added to an EtOAc layer, reduced pressure distilling off of the solvent is carried out after dehydration, and it is

the shape of amorphous. Boc-Lys (Cl-Z) was obtained. ProOBzl HCl 1.7g (7.1 millimol), and HOBt 1.0g (7.7 millimol) and WSCD HCl 1.5g (7.8 millimol) were added to this, it reacted and refined like the example 1, and Boc-Lys(Cl-Z)-ProOBzl was obtained. Acid treatment of the obtained hardening-by-drying object was carried out like the example 1, the Boc radical was removed, Z-Pro 1.7g (6.8 millimol), HOBt 1.0g (7.5 millimol), and WSCD HCl 1.5g (7.8 millimol) were added to the obtained residue, it reacted and refined like the example 1, and oil-like Z-Pro-Lyz(Cl-Bzl)-ProOBzl was obtained. Furthermore, it is sephadex about this. LH-20 column (bore [ of 2.6cm ] x die length of 60cm) It refined, and the fractions which give an almost single spot by TLC were collected, and reduced pressure hardening by drying was carried out. Et<sub>2</sub>O melted to little EtOAc was added to this, and it crystallized by cooling. A crystal is separated, reduced pressure hardening by drying is carried out in a vacuum desiccator, and it is a colorless crystal. 2.68g was obtained. This crystal was dissolved in AcOH 36ml and MeOH 24ml, and all protective groups were removed with the hydrogen catalytic reduction method like the example 1. Sephadex Separation purification was carried out in G-10 column (bore [ of 2.6cm ] x die length of 60cm). When each fraction is checked by TLC (expansion solvent BAPW system R<sub>f</sub>=0.009), the target fraction is collected and condensed and it freeze-dries further, it is the colorless amorphous-like specified substance. 1.4g was obtained. The overall yield so far was 63%. Elemental analysis C<sub>16</sub>H<sub>28</sub>N<sub>4</sub>O<sub>4</sub>MW.340.42 Calculated value C 56.45, H 8.29, N 16.34. Analysis value C54.06, H8.59, N15.27.

[0015] Example 6 [Composition of N-acetylation object (Ac-PKP) of Pro-Lys-Pro]

Amorphous-like object of Pro-Lys-Pro obtained in the example 5 1.2g (3.8 millimol) was dissolved in 10ml of water, N-ASI 1.6g (11.3 millimol) was added after adjusting a solution to pH7, and it stirred at the room temperature overnight. After carrying out concentration hardening by drying of the reaction mixture, residue is dissolved in a small amount of chloroform (CHCl<sub>3</sub>). CHCl<sub>3</sub>: Silica gel produced with the solution of MeOH=10:1 In C-200 column (bore [ of 2.2cm ] x die length of 33cm), by 300ml of solutions of CHCl<sub>3</sub>:MeOH=10:1 after regular placing And it is the fractions 102-115 which are made eluted only in MeOH, carry out fractionation to about 10ml fraction, check each fraction by TLC, and give the spot of the specified substance. eye a collection -- concentration hardening by drying was carried out. Colorless amorphous-like object which will give a single spot (expansion solvent BAPW system R<sub>f</sub>=0.40) by TLC after dissolving this in little water if it freeze-dries 1.25g (76%) It was obtained. It was satisfied with the nuclear-magnetic-resonance spectrum (NMR) of the location of a signal. <sup>1</sup>H NMR (DMSO-d<sub>6</sub>, delta) 1.82 (3H, s) 2.15 (3H, s).

[0016] Example 7 [Composition of Pro-Lys-Pro NH<sub>2</sub> (PKP NH<sub>2</sub>)]

Boc-Lys (Z) 3.5 g (9.2 millimol), Pro NH<sub>2</sub> 1.1g (9.2 millimol), HOBt 1.34g (9.9 millimol), and WSCD HCl 1.95g (10 millimol) are reacted and refined like an example 1, and it is the specified substance. Boc-Lys(Z)-Pro NH<sub>2</sub> was obtained as colorless oily matter. It is Z-Pro 1.5g (6.1 millimol) to the residue which processed like the example 1 and was obtained, HOBt 0.85g (6.3 millimol) and WSCD HCl 1.3g (6.8 millimol) are added, it reacts like the approach indicated in the example 1, Z-Pro-Lys-Pro NH<sub>2</sub> is obtained as oily matter, and it is sephadex like an example 1 about this. What refines in LH-20 column and gives an almost single spot by TLC was obtained. Then, it dissolves in little water after a deprotection reaction with the hydrogen catalytic reduction method using Pd/C 10%, and is sephadex. The specified substance which will give a single spot (expansion solvent BAPW system R<sub>f</sub>=0.17) if it refines and freeze-dries in G-10 column 1.1g was obtained. The overall yield so far was 35%. C<sub>16</sub>H<sub>29</sub>N<sub>5</sub>O<sub>3</sub> MW 339.44 Calculated value C 56.62, H 8.61, N 20.63. Analysis value C 51.56, H 8.83, N 18.63.

[0017] Example 8 [Composition of Pro-Tyr-Pro (PYP)]

Boc-Tyr (Bzl) 2.0g (5.3 millimol), ProOBzl HCl 1.3g (5.38 millimol), HOBt 0.81g (6.0 millimol), and WSCD HCl 1.14g (5.9 millimol) are made to react like an example 1, and it is the specified substance. Boc-Tyr(Bzl)-ProOBzl was obtained. To the residue which carried out acid treatment to this hardening-by-drying object like the example 1, removed the Boc radical, and was obtained Sephadex after dropping Z-Pro 1.3g (5.1 millimol), HOBt 0.76g (5.6 millimol), and WSCD HCl 1.1g (5.6 millimol) and making it react like an example 1 Separation purification was carried out in LH-20 column (bore [ of

2.6cm ] x die length of 60cm). It is little EtOAc about this after carrying out vacuum concentration. It dissolved, Et<sub>2</sub>O was added and it crystallized by cooling. The crystal was separated, reduced pressure hardening by drying was carried out, and 2.68g was obtained. Sephadex after carrying out deprotection of this crystal with a hydrogen catalytic reduction method G-10 The column refined. Dissolved in little MeOH after concentration, add Et<sub>2</sub>O and it was made to crystallize, the crystal after cooling was carried out the \*\* collection, and 1.16g was obtained when it dried. The overall yield so far was 55%. This thing gave the single spot (expansion solvent BAPW system R<sub>f</sub>=0.32) by TLC. Elemental analysis C<sub>19</sub>H<sub>25</sub>N<sub>3</sub>O<sub>5</sub> MW375.43 Calculated value C 60.79, H 6.71, N 11.19. Analysis value C57.54, H6.94, N 10.19.

[0018] Example 9 [Composition of N-acetylation object (Ac-PYP) of Pro-Tyr-Pro]

Add N-Asi 1.1g (8 millimol) to Pro-Tyr-Pro 1g (2.6 millimol) obtained in the example 8, and it is made to react like an example 2, and is chloroform about reaction mixture. The actuation extracted by 20ml was repeated 5 times. After [ dehydration ] reduced pressure hardening by drying of the chloroform layer was carried out by Na<sub>2</sub>SO<sub>4</sub>. Colorless amorphous-like object which dissolves this in little water, freeze-dries, and gives a single spot (expansion solvent BAPW system R<sub>f</sub>=0.51) by TLC 0.68g (61% of yield) was obtained. Elemental analysis C<sub>21</sub>H<sub>27</sub>N<sub>3</sub>O<sub>6</sub> MW417.46 Calculated value C 60.42, H 6.52, N 10.07. Analysis value C 54.52, H 6.58, N 11.04.

[0019] Example 10 [Composition of Pro-Tyr-Pro NH<sub>2</sub> (PYP NH<sub>2</sub>)]

WSCD HCl 1.7g (9.0 millimol) was added to Boc-Tyr (Bzl) 3.25g (8.8 millimol), Pro NH<sub>2</sub> 1.0g (8.8 millimol), and HOBt 1.2g (8.9 millimol), it reacted and refined like the example 1, and Boc-Tyr(Bzl)-Pro NH<sub>2</sub> was obtained as colorless oily matter. Acid treatment of the obtained hardening-by-drying object was carried out like the example 1, the Boc radical was removed, vacuum concentration of the solvent was carried out, Et<sub>2</sub>O was added, and the crystal was deposited. This crystal was separated and reduced pressure hardening by drying was carried out in the vacuum desiccator. Z-Pro 2.0g (7.9 millimol) and HOBt 1.2g (8.9 millimol) were added to the crystal which hardened by drying, WSCD HCl 1.7g (9.0 millimol) was dropped, it reacted and refined like the example 1, and oily matter was obtained. This is dissolved in a small amount of methanol, and it is sephadex. It applied to LH-20 column (bore [ of 2.6cm ] x die length of 60cm), and was eluted with this solvent. After looking for the target fraction in TLC and bringing together in one, reduced pressure hardening by drying is carried out, and it is the amorphous-like specified substance. 4.9g was obtained. Sephadex after performing the deprotection reaction according this to a hydrogen catalytic reduction method The colorless amorphous-like specified substance which freeze-dries after purification in G-10 column, and gives a single spot (expansion solvent BAPW system R<sub>f</sub>=0.42) by TLC 2.9g was obtained. The overall yield so far was 87%. Elemental analysis C<sub>19</sub>H<sub>26</sub>N<sub>4</sub>O<sub>4</sub> MW 377.44 calculated value C 60.95, H 7.60, N14.96. Analysis value C 56.07, H 7.19, N 13.84.

[0020] Example 11 [Composition of N-acetylation of Pro-Tyr-Pro, and C-amidation object (Ac-PYP NH<sub>2</sub>)]

When add N-Asi 1.0g (7 millimol) to PYP NH<sub>2</sub> 1.0g (2.6 millimol) obtained in the example 10, react and refine like an example 2, the fractions which give a single spot (expansion solvent BAPW system R<sub>f</sub>=0.61) mostly by TLC are collected, it condenses and it freeze-dries, it is the colorless amorphous-like specified substance. 1.05g was obtained. The yield so far was 96%. Elemental analysis C<sub>21</sub>H<sub>28</sub>N<sub>4</sub>O<sub>5</sub> MW 416.48 Calculated value C 60.56, H 6.78, N 13.45. Analysis value C 55.11, H 6.01, N 13.09 [0021] Example 12 [Composition of Tyr-Ser-Pro (YSP)]

Having dissolved in DMF 20ml and stirring Boc-Ser (Bzl) 2.0g (6.8 millimol), ProOBzl HCl 1.6g (6.8 millimol), and HOBt 1.0g (7.5 millimol) under ice-cooling, it was made to react like the approach indicated in the example 1 in addition to WSCD HCl 1.4g (7.3 millimol) suspended in DMF 10ml, and Boc-Ser(Bzl)-ProOBzl was obtained. Perform the deBoc radical reaction by acid treatment like the approach indicated in the example 1, add Z-Tyr (Bzl) 2.6g (6.3 millimol) and HOBt 0.85g (6.3 millimol) to the obtained residue, WSCD HCl 1.3g (6.8 millimol) is dropped, it is made to react like an example 1, the obtained residue is melted to little MeOH, and it is sephadex. LH-20 column refined. The target fraction was looked for in TLC, and were collected, and reduced pressure hardening by drying was

carried out. After dissolving this in EtOAc 50ml, 250ml Et<sub>2</sub>O was added and it crystallized by leaving it in a refrigerator. The crystal was separated and reduced pressure hardening by drying was carried out. (2.40g). The deprotection reaction according to this crystal to a hydrogen catalytic reduction method was performed. Sephadex The colorless amorphous-like specified substance which will give a single spot (expansion solvent BAPW system  $R_f=0.30$ ) by TLC if it refines and freeze-dries in G-10 column 1.70g was obtained. The overall yield so far was 68%. Elemental analysis C<sub>17</sub>H<sub>23</sub>N<sub>3</sub>O<sub>6</sub> MW 365.38 Calculated value C 56.03, H 6.63, N 15.37. Analysis value C 55.91, H 6.81, N 13.82.

[0022] Example 13 [Composition of N-acetylation object (Ac-YSP) of Tyr-Ser-Pro]

N-ASI 1.1g (8 millimol) was added to YSP 1.0g (YSP 2.7 millimol) obtained in the example 12, and it was made to react at a room temperature for 2 hours (pH 8 [about]). Sephadex after condensing reaction mixture The specified substance which refines in G-10 column and gives a single spot (expansion solvent BAPW system  $R_f=0.44$ ) by TLC 0.6g was obtained. The overall yield so far was 54%. Elemental analysis C<sub>19</sub>H<sub>25</sub>N<sub>3</sub>O<sub>7</sub> MW 407.41 Calculated value C 56.01, H 6.18, N 10.31. Analysis value C 54.95, H 5.37, N 10.11.

[0023] Example 14 [Composition of Tyr-Ser-Pro NH<sub>2</sub> (YSP NH<sub>2</sub>)]

Added WSCD HCl 2.0g (10 millimol) to Boc-Ser (Bzl) 2.6g (8.8 millimol), Pro NH<sub>2</sub> 1.0g (8.8 millimol), and HOBt 1.30g (9.7 millimol), it was made to react like an example 1, and Boc-Ser(Bzl)-Pro NH<sub>2</sub> was obtained as oily matter. After carrying out acid treatment like the approach which indicated this oily matter in the example 1 and condensing a solvent, Et<sub>2</sub>O was added and precipitation was obtained in the decantation. Reduced pressure hardening by drying of this was carried out, Z-Tyr (Bzl) 3.2g (7.9 millimol) and HOBt 1.2g (8.7 millimol) were added, and WSCD HCl 1.7g (8.8 millimol) suspended in DMF was dropped. It dissolved in little EtOAc after condensing a solvent, Et<sub>2</sub>O was added, and it crystallized by cooling. 3.25g was obtained when reduced pressure drying of the crystal was separated and carried out. When the fraction of the specified substance which performs a deprotection reaction with a hydrogen catalytic reduction method, refines this crystal in G-sephadex 10 column, and gives an almost single spot (expansion solvent BAPW system  $R_f=0.44$ ) by TLC is collected and condensed and it freeze-dries, it is a colorless amorphous-like object. 1.7g was obtained. The overall yield so far was 53%. Elemental analysis C<sub>17</sub>H<sub>24</sub>N<sub>4</sub>O<sub>5</sub> MW 364.39 Calculated value C 56.03, H 6.63, N 15.37. Analysis value C 53.91, H 6.71, N 13.82.

[0024] Example 15 [Composition of N-acetylation of Tyr-Ser-Pro, and C-amidation object (Ac-YSP NH<sub>2</sub>)]

N-ASI 1.0g (7 millimol) was added to YSP NH<sub>2</sub> 0.8g (2.2 millimol) obtained in the example 14, and it was made to react like the approach indicated in the example 2, and processed, and the hardening-by-drying object was obtained. However, since mixing of a condensing agent origin object was presumed by this, it dissolved in little water, and when the extract was repeated 5 times by EtOAc 10ml and the water layer was freeze-dried, 0.87g (yield 95%) of colorless amorphous-like objects was obtained by it. This thing gave the single spot (expansion solvent BAPW system  $R_f=0.56$ ) by TLC.

[0025] Example 16 [Composition of Glu-Arg-Pro (ERP)]

WSCD HCl 2.1g (11.0 millimol) was added to Boc-Arg (NO<sub>2</sub>) 1/2AcOEt 1/4H<sub>2</sub>O 3.7g (10 millimol), ProOBzl HCl 2.4g (10 millimol), and HOBt 1.5g (11 millimol), and it was made to react like an example 1, and processed, and Boc-Arg(NO<sub>2</sub>)-ProOBzl was obtained as oily matter. Acid treatment of the obtained oily matter was carried out like the example 1. Z-Glu(Bzl) 1.7g (5.0 millimol), HOBt 0.75g (5.7 millimol), and WSCD HCl 1.1g (5.5 millimol) were dropped at the obtained oily matter, and it reacted to it like the example 1. After dissolving the obtained residue in little EtOAc, it crystallized, when Et<sub>2</sub>O was added and it cooled. The crystal was separated, reduced pressure hardening by drying was carried out, and the 2.3g crystal was obtained. It is sephadex about the residue which obtained this crystal by carrying out in the clearance reaction of all guard chambers with the hydrogen catalytic reduction method. G-10 column refines, and the fraction which gives a single spot (expansion solvent BAPW system  $R_f=0.007$ ) by TLC is collected and condensed, and it freeze-dries, and is the colorless amorphous-like specified substance. 1.3g was obtained. The overall yield so far was 32%. Elemental analysis C<sub>16</sub>H<sub>28</sub>N<sub>6</sub>O<sub>6</sub> MW 400.44 Calculated value C 47.99, H 7.05, N 20.99. Analysis value C 45.84,



H 7.55, N 19.52.

[0026] Example 17 [Composition of N-acetylation object (Ac-ERP) of Glu-Arg-Pro]

It was obtained in the example 16. N-Asi 1.1g (7.1 millimol) was added to ERP 1.1g (2.7 millimol), and it stirred at the room temperature overnight. Sephadex after condensing reaction mixture G-10 A column refines, and each fraction is collected and condensed, and it freeze-dries, and is the colorless amorphous-like specified substance. 0.80g was obtained. The overall yield so far was 67%. This thing gave the single spot (expansion solvent BAPW system  $R_f=0.16$ ) by TLC. Elemental analysis  $C_{18}H_{30}N_6O_7$  MW 442.47 Calculated value C 48.86, H 6.83, N 18.99. Analysis value C 45.89, H 7.03, N 18.07.

[0027] Example 18 [Composition of C-amidation object (ERP NH<sub>2</sub>) of Glu-Arg-Pro]

WSCD HCl 2.1g (11 millimol) was added to Boc-Arg (NO<sub>2</sub>) 1/2AcOEt 1/4H<sub>2</sub>O 3.7g (10.0 millimol), ProOBzl HCl 2.4g (10 millimol), and HOBt 1.49g (11 millimol), it reacted like the example 1, and chloroform was used and processed instead of EtOAc. It became cloudy when EtOAc was added to the residue which carried out vacuum concentration of the chloroform layer. Furthermore Et<sub>2</sub>O was added, the decantation of the gum-like settlings obtained by leaving it overnight at 4 degrees C was carried out, they were obtained, and reduced pressure hardening by drying was carried out. Acid treatment of the obtained hardening-by-drying object was carried out like the example 1, the Boc radical was removed, the settlings produced during the reaction were obtained by the decantation, and reduced pressure hardening by drying was carried out. After having added Z-Glu (Bzl) 1.7g (5.0 millimol) and HOBt 0.75g (5.5 millimol) to this, dropping WSCD HCl 1.1g (5.7 millimol) and processing by reacting like an example 1, the column refined and colorless oily matter was obtained. Sephadex after performing the clearance reaction of all protective groups for this oily matter with a hydrogen catalytic reduction method G-10 column refined, the fractions of a single spot (expansion solvent BAPW system  $R_f=0.21$ ) were mostly collected by TLC, and crystalline residue was obtained. Cold MeOH was added to this, and 0.2g was obtained when the \*\* collection of the crystal was unfolded and carried out. The overall yield so far was 6%. Elemental-analysis  $C_{16}H_{29}N_7O_5$  MW 399.45 Calculated value C 48.11, H 7.32, N 24.55. Analysis value C 46.61, H 6.92, N 22.34.

[0028] Example 19 [Composition of Pro-Thr-Trp (PTW)]

the DMF solution of Boc-ThrOSu 3.7g (12 millimol) and TrpOBzl HCl 3.8g (12 millimol) -- the bottom of ice-cooling stirring, and TEA -- in addition, it was made to react after checking that it is pH 7-8 overnight After treatment was carried out like the example 1, and Boc-Thr-TrpOBzl was obtained as oily matter. Then, acid treatment was carried out like the example 1, Z-Pro 2.8g (11 millimol) was added to the residue which might be removed in the Boc radical, the DMF 10ml solution of DCCD 2.5g (12 millimol) was dropped, and it was made to react at 4 degrees C overnight. Vacuum concentration of the filtrate is carried out the back according to \*\*, settlings are processed like an example 1, and it is an amorphous-like object. 4.21g was obtained. Among these, the residue which removed 2g with the hydrogen catalytic reduction method, and was obtained in the protective group is dissolved in little water, and it is sephadex. The fractions which refine in G-10 column and give a single spot (expansion solvent BAPW system  $R_f=0.33$ ) mostly by TLC were collected, and reduced pressure hardening by drying was carried out. Little MeOH is added to residue, it was crystallized, after cooling, the crystal was separated, it dried and 1.40g was obtained. The overall yield so far was 30%. Elemental analysis  $C_{20}H_{25}N_4O_5$  MW 402.22 Calculated value C 59.69, H 6.51, N 13.91. Analysis value C 55.90, H 6.41, N 12.75.

[0029] Example 20 [Composition of Pro-Thr-Trp NH<sub>2</sub> (PTW NH<sub>2</sub>)]

Boc-ThrOSu 6.0g (19 millimol) is dissolved in DMF 50ml, and it is Trp NH<sub>2</sub> hydrochloride. 4.6g (19 millimol) and HOBt 2.9g (21 millimol) were added with powder, and the stirring dissolution was carried out. After adding TEA under ice-cooling stirring and checking that it is pH 7-8 WSCD HCl 4.1g (21 millimol) The DMF 10ml solution was added and it was made to react at 4 degrees C overnight. Precipitate is \*\*\*\*(ed) and it is filtrate after vacuum concentration. EtOAc 500ml was added and 8.3g (87%) was obtained by processing like an example 1 by making specified substance Boc-Thr-Trp NH<sub>2</sub> into colorless oily matter. This is made to react like an example 1, and it processes, and is oily matter.

7.2g (16 millimol, 98%) was obtained. To then, this Z-Pro 4.1g (16 millimol), HOBt 2.5g (18 millimol), and WSCD HCl 3.5g (18 millimol) were added, and it was made to react like an example 1, it processed, and 7.3g (70%) was obtained by using Z-Pro-Thr-Trp NH<sub>2</sub> as an amorphous-like object. This amorphous-like object Pd/C 1g was added to 2.5g 10%, and the deprotection reaction was performed after a reaction / processing like the example 1. What was obtained is dissolved in little water and it is sephadex. The specified substance which will consist of a single spot (expansion solvent BAPW system R<sub>f</sub>=0.47) if it refines and freeze-dries in G-10 column 1.0g was obtained. Elemental analysis C<sub>20</sub>H<sub>27</sub>N<sub>5</sub>O<sub>4</sub> MW.401.33 Calculated value C 59.80, H 6.78, N 17.46. Analysis value C 57.63, H 6.94, N 16.27.

[0030] Example 21 [Composition of N-acetylation object (Ac-PTW NH<sub>2</sub>) of Pro-Thr-Trp NH<sub>2</sub>] After adding N-ASI 0.9g (6 millimol) to PTW NH<sub>2</sub> 0.95g (2.4 millimol) obtained in the example 20 and making it react at a room temperature overnight, concentration hardening by drying was carried out under reduced pressure. Silica gel which melted with a small amount of chloroform, and was produced by the mixture of chloroform:methanol =30:1 Regular placing is carried out to C-200 (Wako Pure Chem make) column (bore [ of 2.6cm ] x die length of 45cm), and it is chloroform:methanol =30:1 mixture. 400ml, 10:1 mixture Only 300ml and a methanol were made eluted in 500ml, and each fractions (about 10ml) were collected even for fractions 72-75 after the check by TLC. When residue is dissolved in little water and it freeze-dries after concentration hardening by drying, it is a colorless amorphous-like object. 0.8g was obtained. The overall yield so far is 75%, and is \*\*\*\*. This thing gave the single spot (expansion solvent BAPW system R<sub>f</sub>=0.64) by TLC. Elemental analysis C<sub>22</sub>H<sub>29</sub>N<sub>5</sub>O<sub>3</sub> MW443.50 Calculated value C 59.58, H 6.59, N 15.79. Analysis value C 56.98, H 6.54, N 15.34. 3g was obtained.

[0031] Example 22 [Composition of thioPro-Thr-Trp (P'TW)] the DMF solution of Boc-ThrOSu 3.7g (12 millimol) and TrpOBzl HCl 3.8g (12 millimol) -- the bottom of ice-cooling stirring, and TEA -- in addition, it was made to react after checking that it is pH 7-8 overnight After treatment was carried out like the example 1, and Boc-Thr-TrpOBzl was obtained as oily matter. Then, acid treatment was carried out like the example 1, the Boc radical was removed, Boc-thioPro2.8g (12 millimol) was added to the obtained residue, the DMF 10ml solution of HOBt 1.784g (13.2 millimol) and WSCD HCl 2.53g (13.2 millimol) was dropped, and it was made to react at 4 degrees C overnight. Vacuum concentration of the filtrate is carried out the back according to \*\*, settlings are processed like an example 1, and it is an amorphous-like object. 6.3g was obtained. Cutting of the protective group by HF of Boc-thioPro-Thr-TrpOBzl 6.3g obtained by coupling went as follows. Boc-thioPro-Thr-TrpOBzl 6.3g was equally divided into ten, and HF cut, respectively. That is, Boc-thioPro-Thr-TrpOBzl is taken in a reaction container, and it is thioanisole. 11ml was added and it put on the room temperature for 1 hour. It was made to react, stirring a reaction container by the dry ice-acetone bath and stirring HF 100ml at installation and 0 degree C under cooling for 1 hour. Reduced pressure clearance of the HF was carried out with the stream aspirator, and it was made to dry with a vacuum pump further. The obtained residue was dissolved by 100ml of acetic-acid water solutions 10%, and the thioanisole which washes twice and remains with 50ml diethylether was removed. The acetic-acid water-solution fraction was neutralized, 3.2g of things which carried out concentration hardening by drying was dissolved in little chloroform-methanol mixture (5:1), the silica gel column chromatography refined, and the specified substance was obtained. Yield of 0.6g. TLC (BAPW system R<sub>f</sub>=0.62) gave the single spot. Elemental analysis C<sub>19</sub>H<sub>25</sub>N<sub>4</sub>O<sub>5</sub>S MW420.53 Calculated value C 54.22, H 5.99, N 13.35. Analysis value C 55.90, H 6.41, N 12.75.

[0032] Example 23 [Composition of D-Pro-Thr-Trp NH<sub>2</sub> (D-PTW NH<sub>2</sub>: Pro is D object)] the DMF solution of Boc-ThrOSu 3.7g (12 millimol) and TrpOBzl HCl 3.8g (12 millimol) -- the bottom of ice-cooling stirring, and TEA -- in addition, it was made to react after checking that it is pH 7-8 overnight After treatment was carried out like the example 1, and Boc-Thr-TrpOBzl was obtained as oily matter. Then, acid treatment was carried out like the example 1, the Boc radical was removed, Z-D-Pro 2.8g (11 millimol) was added to the obtained residue, the DMF 10ml solution of DCCD 2.5g (12 millimol) was dropped, and it was made to react at 4 degrees C overnight. Vacuum concentration of the filtrate is carried out the back according to \*\*, settlings are processed like an example 1, and it is an



amorphous-like object. 4.21g was obtained. Among these The fractions which dissolve the residue which removed the protective group with the catalytic reduction method, and was obtained in 2g in little water, refine in G-sephadex 10 column, and give a single spot mostly by TLC were collected, and reduced pressure hardening by drying was carried out. Little MeOH is added to residue, it was crystallized, after cooling, the crystal was separated, it dried and 1.40g (30% of overall yields) was obtained (BAPW system  $R_f=0.33$ ). Elemental analysis  $C_{20}H_{25}N_4O_5$  MW 402.22 Calculated value C 59.69, H 6.51, N 13.91. Analysis value C 55.90, H 6.41, N 12.75.

[0033] Example 24 [Composition of Glu-D-Arg (E-D-R: Arg is D object)]

Added WSCD HCl 2.1g (11 millimol) to DMF 100ml under the dissolution and ice-cooling, Z-Glu(Bzl) OH 3.4g (10 millimol), D-Arg(NO<sub>2</sub>) OBzl Tos 4.82g (10 millimol), and HOBt 1.5g (11 millimol) were made to react like an example 1, after treatment was performed, and 5.3g (8 millimol) of oily matter of Z-Glu(Bzl)-D-Arg(NO<sub>2</sub>) OBzl was obtained. The deprotection reaction was performed with the catalytic reduction method, G-sephadex 10 column refined this oily matter, the fractions of the specified substance which gives an almost single spot by TLC were collected, and concentration and freeze drying were performed. 1.82g (6 millimol) was obtained as a colorless amorphous-like object (TLC: BAPW system  $R_f=0.58$ ). Elemental analysis  $C_{11}H_{21}N_5O_5$  and MW 303.33 Calculated value C 43.52, H 6.92, N 23.14. Analysis value C 42.98, H 6.88, N 22.76 [0034] Example of a trial 1 [Focus formation inhibition trial]

Put in the NIH 3T3 cell which carried out the transformation to 8 hole love tech chamber (slide glass size) by proto oncogene erbB-2, and consider one hole as control. A sample is added in remnants and seven holes and it cultivates with a carbon-dioxide-gas incubator 5 37-degree C%, and every 24 hours, for five days, a cell proliferation condition (the number of focuses) is observed with an optical microscope, and a part of the (the sample last concentration 250microg/ml) result a photograph of was taken each time is shown in a table 1 (table 1).

[0035]

[A table 1]

表1 フォーカスアッセイ

サンプル (実施例番号) \ 日数	フォーカス数				
	1	2	3	4	5
9	0	0	0	2	6
13	0	1	1	4	5
17	0	0	0	3	17

[0036] Example of a trial 2 [MTT measuring method]

The NIH 3T3 cell which cancerated by various oncogenes to the DME culture medium which contains a cow blood serum 10% was suspended so that it might become with  $5 \times 10^4$  cells / ml, and the 100microl was poured distributively to each well of 96 hole flat bottom plate. Among these, only the culture medium of tales doses was added to three wells, and it considered as electronegative control. This plate was added to 880microg [/ml ] concentration in 37 degrees C and 5% carbon-dioxide-gas incubator, and for example, water, and the tripeptide and 10micro of its derivative solution I of this invention prepared with organic solvents, such as an acetone, dimethylformamide, dimethyl sulfoxide, and alcohol, were added to each well after culture overnight (80microg [/ml ] final concentration). MTT of 5mg [/ml ] concentration after 2-hour culture and 10micro of PBS (-) solutions I were added to all wells in 37 degrees C and 5% carbon-dioxide-gas incubator, and this was cultivated within 37 degrees C and 5% carbon-dioxide-gas incubator for 2 hours. The at-long-intervals alignment of each plate was carried out



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TECHNICAL FIELD

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[Field of the Invention] This invention relates to the remedy constituent containing tripeptide or a dipeptide useful as an anticancer agent.

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[Translation done.]

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PRIOR ART

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[Description of the Prior Art] Research of the inhibitor of thrombin activity is known about using the tripeptide and its derivative as drugs (journal medical chemistry: vol.37, p.2122, 1994 year \*\*:vol.36, p. 1993 [ 300 or ]). Moreover, the development research as drugs of a comparatively short peptide and its derivative is seen by for example, a \*\*\*\*\* No. 502154 [ four to ] official report, a \*\*\*\*\* No. 502306 [ four to ] official report, a \*\*\*\*\* No. 502308 [ four to ] official report, a \*\*\*\*\* No. 502309 [ four to ] official report, etc. In these researches, a prolyl radical is contained in the amino acid sequence of the peptide, and the number of amino acid of the die length of an array is five or more. These peptides can also be said to be the TNF amelioration peptide guided from the tumor necrosis factor (TNF). Thus, some researches which use a short peptide as drugs were made. that intracellular is easy to be incorporated considers a short peptide enough -- having -- in addition -- and since it is decomposed in the living body and they serve as harmless amino acid, it is thought that the side effect over administration to a living body is hardly produced. Therefore, it is thought that these short peptides begin cancer and can serve as promising drugs as a remedy of other diseases in the future. However, about the utilization as an anticancer agent of a short peptide, it was not known at all until now.

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[Translation done.]

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EFFECT OF THE INVENTION

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[Effect of the Invention] The completely new remedy constituent which contains tripeptide by this invention is offered. The active principle of the remedy constituent of this invention is a peptide, it is decomposed into amino acid and the object is metabolized in the living body. Therefore, there are very few dangers of causing a side effect, and the remedy constituent of this invention has them as drugs, when a living body is medicated. [ effective ]

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TECHNICAL PROBLEM

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[Problem(s) to be Solved by the Invention] While this invention persons investigated the growth mechanism of a cancer cell, they came to get to know that the protein-protein interaction has determined the important metabolic fate. Although it was generally thought that the complicated structure of the protein itself was participating in association with protein and protein, the result of detailed research of the joint domain of a protease and its inhibitor showed that the peptide chain with a very short part of the protein of big molecular weight was participating in proteinic association. When making the peptide equivalent to the joint domain of the protein which participates in growth of a cancer cell from the above thing live together in intracellular, association with this protein and other protein could be prevented, and since it was possible to control the function of this protein as a result, it was thought that such a short peptide was useful as an anticancer agent. The object of this invention is to offer the short peptide which has the carcinostatic effectiveness, and the object of this invention still more specifically has it in offering the remedy constituent containing the tripeptide which has a specific amino acid sequence, dipeptides, or those derivatives, and it is for other objects of this invention to offer the remedy constituent containing the salt accepted physiologically [ the tripeptide which has a specific amino acid sequence, a dipeptide, or its derivative ].

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[Translation done.]

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MEANS

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[Means for Solving the Problem] The various oncogene products as an amino acid sequence of tripeptide with the promising this invention persons, For example K-Sam, Yes, Ret, Kit and Fms, ErbB, Met, Ros, Sea, Trk, Src, Fgr, Fyn, Lyn, The consensus sequence of the oncogene product [ Arg / Lck, Hck, Abl, ] which has the array further called the Sark homology (SH) domain was searched, and the array which participates in proteinic association through research of the tripeptide equivalent to a joint domain etc. was predicted. Consequently, it finds out having the effectiveness that the tripeptide which has a fixed amino acid sequence checks growth of a cancer cell, and came to complete this invention based on this knowledge.

[0005] Namely, the tripeptide this invention is indicated to be by Pro-A-B (however, A=Phe, Lys, Asn and Tyr, Thr; B=Pro, and Trp are shown.), Or the remedy constituent which contains those salts accepted physiologically as an active principle, The tripeptide shown by Thioproline-Thr-Trp (Following thioPro or P'), Or the remedy constituent which contains those salts accepted physiologically as an active principle, The dipeptide shown by Glu-Arg, or the remedy constituent which contains those salts accepted physiologically as an active principle, The remedy constituent which contains the tripeptide shown by C-D-Pro (however, C=Tyr, Glu, Pro; D=Asn, and Ser, Arg and Tyr are shown) or those salts that were accepted physiologically as an active principle is offered.

[0006]

[Embodiment of the Invention] As an amino acid sequence of the tripeptide concerning this invention, they are Pro-Phe-Pro, Pro-Lys-Pro, Pro-Tyr-Pro, Tyr-Ser-Pro, Glu-Arg-Pro, Pro-Tyr-Trp, etc. As an amino acid sequence of the dipeptide concerning this invention, it is Glu-Arg etc. In this invention, that by which the amino acid of the amino terminal of the above-mentioned tripeptide and a dipeptide was acylated, the thing by which the amino acid of a C terminal was amidated, and the thing with which ends were embellished like the above are also used. As an N-acylation derivative of the tripeptide of this invention, and a dipeptide, it is a formyl group, an acetyl group, an aryl carbonyl group, and an aromatic series carbonyl group derivative, and the amino group, an alkylamino radical, and an aromatic series amino-group derivative can be raised as a C-amidation derivative. As a salt accepted physiologically [ the tripeptide of this invention, a dipeptide, and its derivative ], a hydrochloric acid, a citric acid, a phosphoric acid, a tartaric acid, a lactic acid, an acetic acid, a formic acid, a fumaric acid, a maleic acid, a succinic acid, etc. are raised.

[0007] Composition of the tripeptide concerning this invention, a dipeptide, and its derivative is the Japanese Biochemical Society edit \*\*\*\*\* experiment lecture 2. As proteinic chemistry (below) has a publication, it is easily compoundable by any approach of a solid phase technique or a liquid phase process. Purification of the tripeptide concerning this invention, a dipeptide, and its derivative can be performed by the usual approaches, such as a silica gel column chromatography and an ion-exchange column chromatography. There is no definition also in an eluate and it can use the usual organic solvents, such as water, a methanol, and chloroform, for it. It is 1-ethyl about the amino acid of the commercial item which protected the carboxyl group by benzyl (Bzl radical) first when describing composition of the tripeptide concerning this invention, a dipeptide, and its derivative in more detail. - 3

-(3-dimethylaminopropyl)- It condenses using condensing agents, such as a carbodiimide hydrochloride (WSCD HCl) and dicyclohexylcarbodiimide (DCCD), and a dipeptide is obtained. As a solvent used for the condensation reaction of the tripeptide of this invention, and a dipeptide, N.N-dimethylformamide (DMF), dimethyl sulfoxide (DMSO), an acetonitrile, 1,4-dioxane, tetrahydro furans (THF), and such mixture are suitable. Clearance of the protective group of tripeptide and a dipeptide can use the hydrogen catalytic reduction method or liquefaction hydrogen fluoride (HF) which made the catalyst a trifluoro methansulfonic acid method (TMSFA law), and palladium/carbon. Generally as a solvent used with a hydrogen catalytic reduction method, it is used, and is satisfactory. A methanol (it may outline Following MeOH), acetic acids, or such mixture are suitable. The general approach of thin-layer chromatography (TLC) can be used for the reaction progress in each synthetic step, or the check of purity. In an expansion solvent, the check of a spot can use the hydrogen bromide (HBr)-ninhydrin method using a chloroform-methanol system and an n-butanol-acetic-acid-pyridine-water (4:1:1:2) system.

[0008] Although there is especially no constraint except the remedy constituent of this invention containing the tripeptide obtained as mentioned above, a dipeptide and its derivative, or those salts that were accepted physiologically as an active principle, the additive usually used on the occasion of pharmaceutical-preparation-izing of a compound may also be included. The remedy constituent of this invention may be used as an anticancer agent which has the outstanding activity. Adapted \*\*\*\* are leukemia, an osteosarcoma, a breast cancer, an ovarian cancer, gastric cancer, colon cancer, lung cancer, etc. Although the medication method of the remedy constituent of this invention naturally changes with symptoms for administration, it divides and medicates 1 time or several times with the adult 1 person 1 Japanese hits 0.01-10g. In addition, the side effect of the remedy constituent of this invention is satisfactory in any way in anticipated-use extent.

[0009] The judgment of the carcinostatic effectiveness can add the tripeptide and its derivative, or these salts that were accepted physiologically of this invention to the culture medium of the culture cell strain which cancerated by various oncogenes, and can observe change of the gestalt of a cell, a reproductive rate, a survival rate, etc. by cultivating under the usual condition. For example, it is convenient if you use 3-[4 and 5-dimethyl thiazole-2-IRU]-2 and 5 diphenyl-tetrazolium bromine salt method (the MTT method) for reproductive rate depressor effect measurement of a cancer cell. Culture cell strains which cancerated as a cancer cell stock by oncogenes, such as v-Ha-ras, Ki-ras, v-src, c-erbB -2, v-mos, and SV40, such as NIH3 T3 fibroblast and 3Y1 rat fibroblast, can be tried. This invention is not limited by this although an example explains this invention concretely below.

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[Translation done.]



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EXAMPLE

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[Example]

Example 1 [Composition of Pro-Phe-Pro (PFP)]

Boc-Phe 2.7g (10 millimol), ProOBzl HCl 2.4g (10 millimol), and 1-hydroxy benzotriazol (HOBt) 1.5g (11 millimol) were dissolved in DMF 30ml, and WSCD HCl 1.7g (11 millimol) suspended in DMF 10ml was dropped at the bottom of ice-cooling stirring. triethylamine (TEA) -- after adding 1.8ml and checking that it is pH 7-8 with the omnipotent pH indicator paper, it stirred at the room temperature all night. After filtering and removing the precipitation which deposited and carrying out vacuum concentration of the filtrate, 300 ml ethyl acetate (EtOAc) was added and it washed in order of 10% salt (NaCl) water solution of 4% sodium hydrogencarbonate (NaHCO<sub>3</sub>), 10%NaCl water solution, 10% NaCl water solution of 0.4M citric acid, and 10%NaCl water solution. Anhydrous sodium sulfate (Na<sub>2</sub>SO<sub>4</sub>) was added to the EtOAc layer, reduced pressure distilling off of the solvent was carried out after dehydration, and reduced pressure hardening by drying of the residue was carried out in the desiccator. It is 4 Ns to this hardening-by-drying object. A hydrochloric acid/dioxane 40ml, thioanisole 4ml was added and it reacted under ice-cooling for 2 hours. Dioxane after carrying out vacuum concentration of this The actuation which adds and condenses 50ml was repeated twice, and the actuation which adds and condenses diethylether (Et<sub>2</sub>O) similarly was repeated twice. Z-Pro 1.5g (6.2 millimol) and HOBt 0.84g (6.8 millimol) were added to the hardening-by-drying object which carried out reduced pressure hardening by drying of this, and it dissolved in it at DMF 30ml. WSCD HCl 1.2g (6.8 millimol) suspended in DMF 10ml was dropped under ice-cooling stirring. After preparing to pH 7-8 by TEA 1.6ml, it was made to react at a room temperature for 1.5 hours. After making it react at 4 more degrees C all night, the precipitation which deposited was filtered and removed and vacuum concentration of the filtrate was carried out. EtOAc 300ml was added and it washed in order of 10% NaCl water solution of 4%NaHCO<sub>3</sub>, 10%NaCl water solution, 10%NaCl water solution of 0.4M citric acid, and 10%NaCl water solution. Anhydrous Na<sub>2</sub>SO<sub>4</sub> was added to the EtOAc layer, reduced pressure distilling off of the solvent was carried out after dehydration, and reduced pressure hardening by drying of the residue was carried out in the desiccator. A hardening-by-drying object is dissolved in a small amount of methanol (MeOH), and it is sephadex. Separation purification was carried out in LH-20 column (bore [ of 3cm ] x die length of 55cm). It was eluted with this solvent and fractionation was carried out in 5 minutes (about 8ml) per one fraction. By TLC (the system of chloroform-MeOH was used for the Kieselgel 60F 254 expansion solvent.), after the check, the fractions containing the specified substance were collected and reduced pressure hardening by drying was carried out about each fraction. This was dissolved in the mixed liquor of MeOH 20ml and a 20 ml acetic acid (AcOH), and it stirred under the nitrogen air current. It is stirring A stop and 10% After adding palladium / carbon (Pd/C) (51.60% hydrated compound) 2.0g and leading a hydrogen air current, it stirred violently by ordinary temperature ordinary pressure, and the reaction was started. After it permuted the stop in the place which absorption of hydrogen ended and nitrogen gas permuted hydrogen gas for stirring, the catalyst was carried out the \*\* exception. Water was added to what carried out vacuum concentration of the filtrate, and vacuum concentration was carried out once again. It is sephadex about a concentrate. Separation

purification was carried out in G-10 column (bore [ of 2.6cm ] x die length of 60cm). It was eluted with water and fractionation was carried out in 5 minutes (about 10ml) per one fraction. The purity of each fraction was checked by TLC (n-butanol:acetic-acid:pyridine:water =4:1:1:2 (BAPW system) was used for the expansion solvent.) (Rf value 0.39), the fractions which give the spot of the specified substance individually were collected, and reduced pressure hardening by drying was carried out. This hardening-by-drying object is dissolved in 15ml water, and it freeze-dries, and is the colorless amorphous-like specified substance. 1.0g It obtained. The overall yield so far was 28%. Elemental analysis C<sub>19</sub>H<sub>25</sub>N<sub>3</sub>O<sub>4</sub> MW.359.43 Calculated value C 63.49, H 7.01, N 11.69. Analysis value C 59.41, H 7.42, N 10.66.

[0011] Example 2 [Composition of N-acetylation object (Ac-PFP) of Pro-Phe-Pro]

Pro-Phe-Pro 1.2g (3.3 millimol) obtained in the example 1 is dissolved in 10ml of water, and it is this. N-acetyl succinimide (N-ASI) 1.5g (10 millimol) is added, and it is 1 N. pH was stirred at the room temperature after preparation to about 7 with the potassium-hydroxide water solution all night. Sephadex after condensing reaction mixture Separation purification was carried out in G-10 column (bore [ of 2.6cm ] x die length of 60cm). The colorless amorphous-like specified substance which will give a single spot (expansion solvent BAPW system Rf=0.56) by TLC if each fraction is checked by TLC and concentration hardening by drying is carried out 1.2g (90% of yield) was obtained. Elemental analysis C<sub>21</sub>H<sub>27</sub>N<sub>3</sub>O<sub>5</sub> MW.401.46 Calculated value C 62.83, H 6.78, N 10.47. Analysis value C 59.69, H 6.50, N 10.97.

[0012] Example 3 [Composition of C-amidation object (PFP NH<sub>2</sub>) of Pro-Phe-Pro]

Boc-Phe 2.3g (8.8 millimol), Pro NH<sub>2</sub> 1.0g (8.8 millimol), and HOBt 1.33g (9.8 millimol) were dissolved in DMF 30ml, and it suspended in DMF 10ml under ice-cooling stirring. WSCD HCl (MW115.24 36.46) 1.7g (8.8 millimol) was dropped. After checking that it is pH 7-8 with the omnipotent pH indicator paper [ TEA 1.7ml ], it was made to react at 4 degrees C all night. It processed like the example 1 after the reaction, and Boc-Phe-Pro NH<sub>2</sub> was obtained as colorless oily matter. It is 4 Ns to this. A hydrochloric acid/dioxane 16ml, thioanisole 1.5ml was added and it stirred under ice-cooling for 1 hour. It processed like the example 1, Z-Pro 2.0g (7.9 millimol) and HOBt 1.1g (8.8 millimol) were added to the obtained residue, and it dissolved in DMF 20ml. WSCD HCl 1.7g (8.7 millimol) suspended in DMF 10ml was dropped under ice-cooling stirring. After preparing to pH 7-8 by TEA 1.5ml, it was made to react at 4 degrees C all night. The same processing as an example 1 was performed after the reaction, and the oily matter of Z-Pro-Phe-Pro NH<sub>2</sub> was obtained. It is sephadex like an example 1. LH-20 column refines, and by TLC, a single spot is obtained mostly, it continues, and they are after a deprotection reaction and sephadex by the hydrogen catalytic reduction method. G-10 column refines, and it is made to crystallize from Et<sub>2</sub>O, and is a colorless crystal. 0.2g (BAPW system Rf=0.48) was obtained. The overall yield so far was 39%. Elemental analysis C<sub>19</sub>H<sub>26</sub>N<sub>4</sub>O<sub>3</sub> MW.358.44 Calculated value C 60.37, H 7.31, N 15.63. Analysis value C 60.48, H 7.38, N 14.55.

[0013] Example 4 [Composition of N-acetylation of Pro-Phe-Pro, and C-amidation object (Ac-PFP NH<sub>2</sub>)] It is the specified substance by adding 6ml of water, dissolving in PFP NH<sub>2</sub> 1.0g (2.8 millimol) obtained in the example 3, and reacting and refining like an example 2. 0.9g (80% of yield) was obtained. This gave the single spot (expansion solvent BAPW system Rf=0.62) by TLC. Elemental analysis C<sub>21</sub>H<sub>28</sub>N<sub>4</sub>O<sub>4</sub> MW.400.25 Calculated value C 62.98, H 7.05, N 13.99. Analysis value C 58.91, H 6.68, N 13.01.

[0014] Example 5 [Composition of Pro-Lys-Pro (PKP)]

It dissolved in 400ml EtOAc and Boc-Lys (Cl-Z) t-Bu NH<sub>2</sub> 3.5g (7.1 millimol) was washed in order of 10%NaCl water solution of 0.4M citric acid, and 10%NaCl water solution. Anhydrous Na<sub>2</sub>SO<sub>4</sub> is added to an EtOAc layer, reduced pressure distilling off of the solvent is carried out after dehydration, and it is the shape of amorphous. Boc-Lys (Cl-Z) was obtained. ProOBzl HCl 1.7g (7.1 millimol), and HOBt 1.0g (7.7 millimol) and WSCD HCl 1.5g (7.8 millimol) were added to this, it reacted and refined like the example 1, and Boc-Lys(Cl-Z)-ProOBzl was obtained. Acid treatment of the obtained hardening-by-drying object was carried out like the example 1, the Boc radical was removed, Z-Pro 1.7g (6.8 millimol), HOBt 1.0g (7.5 millimol), and WSCD HCl 1.5g (7.8 millimol) were added to the obtained

residue, it reacted and refined like the example 1, and oil-like Z-Pro-Lyz(Cl-Bzl)-ProOBzl was obtained. Furthermore, it is sephadex about this. LH-20 column (bore [ of 2.6cm ] x die length of 60cm) It refined, and the fractions which give an almost single spot by TLC were collected, and reduced pressure hardening by drying was carried out. Et<sub>2</sub>O melted to little EtOAc was added to this, and it crystallized by cooling. A crystal is separated, reduced pressure hardening by drying is carried out in a vacuum desiccator, and it is a colorless crystal. 2.68g was obtained. This crystal was dissolved in AcOH 36ml and MeOH 24ml, and all protective groups were removed with the hydrogen catalytic reduction method like the example 1. Sephadex Separation purification was carried out in G-10 column (bore [ of 2.6cm ] x die length of 60cm). When each fraction is checked by TLC (expansion solvent BAPW system R<sub>f</sub>=0.009), the target fraction is collected and condensed and it freeze-dries further, it is the colorless amorphous-like specified substance. 1.4g was obtained. The overall yield so far was 63%. Elemental analysis C<sub>16</sub>H<sub>28</sub>N<sub>4</sub>O<sub>4</sub>MW.340.42 Calculated value C 56.45, H 8.29, N 16.34. Analysis value C54.06, H8.59, N15.27.

[0015] Example 6 [Composition of N-acetylation object (Ac-PKP) of Pro-Lys-Pro]

Amorphous-like object of Pro-Lys-Pro obtained in the example 5 1.2g (3.8 millimol) was dissolved in 10ml of water, N-ASI 1.6g (11.3 millimol) was added after adjusting a solution to pH7, and it stirred at the room temperature overnight. After carrying out concentration hardening by drying of the reaction mixture, residue is dissolved in a small amount of chloroform (CHCl<sub>3</sub>). CHCl<sub>3</sub>: Silica gel produced with the solution of MeOH=10:1 In C-200 column (bore [ of 2.2cm ] x die length of 33cm), by 300ml of solutions of CHCl<sub>3</sub>:MeOH=10:1 after regular placing And it is the fractions 102-115 which are made eluted only in MeOH, carry out fractionation to about 10ml fraction, check each fraction by TLC, and give the spot of the specified substance. eye a collection -- concentration hardening by drying was carried out. Colorless amorphous-like object which will give a single spot (expansion solvent BAPW system R<sub>f</sub>=0.40) by TLC after dissolving this in little water if it freeze-dries 1.25g (76%) It was obtained. It was satisfied with the nuclear-magnetic-resonance spectrum (NMR) of the location of a signal. <sup>1</sup>H NMR (DMSO-d<sub>6</sub>, delta) 1.82 (3H, s) 2.15 (3H, s).

[0016] Example 7 [Composition of Pro-Lys-Pro NH<sub>2</sub> (PKP NH<sub>2</sub>)]

Boc-Lys (Z) 3.5 g (9.2 millimol), Pro NH<sub>2</sub> 1.1g (9.2 millimol), HOBt 1.34g (9.9 millimol), and WSCD HCl 1.95g (10 millimol) are reacted and refined like an example 1, and it is the specified substance. Boc-Lys(Z)-Pro NH<sub>2</sub> was obtained as colorless oily matter. It is Z-Pro 1.5g (6.1 millimol) to the residue which processed like the example 1 and was obtained, HOBt 0.85g (6.3 millimol) and WSCD HCl 1.3g (6.8 millimol) are added, it reacts like the approach indicated in the example 1, Z-Pro-Lys-Pro NH<sub>2</sub> is obtained as oily matter, and it is sephadex like an example 1 about this. What refines in LH-20 column and gives an almost single spot by TLC was obtained. Then, it dissolves in little water after a deprotection reaction with the hydrogen catalytic reduction method using Pd/C 10%, and is sephadex. The specified substance which will give a single spot (expansion solvent BAPW system R<sub>f</sub>=0.17) if it refines and freeze-dries in G-10 column 1.1g was obtained. The overall yield so far was 35%. C<sub>16</sub>H<sub>29</sub>N<sub>5</sub>O<sub>3</sub> MW 339.44 Calculated value C 56.62, H 8.61, N 20.63. Analysis value C 51.56, H 8.83, N 18.63.

[0017] Example 8 [Composition of Pro-Tyr-Pro (PYP)]

Boc-Tyr (Bzl) 2.0g (5.3 millimol), ProOBzl HCl 1.3g (5.38 millimol), HOBt 0.81g (6.0 millimol), and WSCD HCl 1.14g (5.9 millimol) are made to react like an example 1, and it is the specified substance. Boc-Tyr(Bzl)-ProOBzl was obtained. To the residue which carried out acid treatment to this hardening-by-drying object like the example 1, removed the Boc radical, and was obtained Sephadex after dropping Z-Pro 1.3g (5.1 millimol), HOBt 0.76g (5.6 millimol), and WSCD HCl 1.1g (5.6 millimol) and making it react like an example 1 Separation purification was carried out in LH-20 column (bore [ of 2.6cm ] x die length of 60cm). It is little EtOAc about this after carrying out vacuum concentration. It dissolved, Et<sub>2</sub>O was added and it crystallized by cooling. The crystal was separated, reduced pressure hardening by drying was carried out, and 2.68g was obtained. Sephadex after carrying out deprotection of this crystal with a hydrogen catalytic reduction method G-10 The column refined. Dissolved in little MeOH after concentration, add Et<sub>2</sub>O and it was made to crystallize, the crystal after cooling was carried

out the \*\* collection, and 1.16g was obtained when it dried. The overall yield so far was 55%. This thing gave the single spot (expansion solvent BAPW system  $R_f=0.32$ ) by TLC. Elemental analysis  $C_{19}H_{25}N_3O_5$  MW375.43 Calculated value C 60.79, H 6.71, N 11.19. Analysis value C57.54, H6.94, N 10.19.

[0018] Example 9 [Composition of N-acetylation object (Ac-PYP) of Pro-Tyr-Pro]

Add N-ASI 1.1g (8 millimol) to Pro-Tyr-Pro 1g (2.6 millimol) obtained in the example 8, and it is made to react like an example 2, and is chloroform about reaction mixture. The actuation extracted by 20ml was repeated 5 times. After [ dehydration ] reduced pressure hardening by drying of the chloroform layer was carried out by  $Na_2SO_4$ . Colorless amorphous-like object which dissolves this in little water, freeze-dries, and gives a single spot (expansion solvent BAPW system  $R_f=0.51$ ) by TLC 0.68g (61% of yield) was obtained. Elemental analysis  $C_{21}H_{27}N_3O_6$  MW417.46 Calculated value C 60.42, H 6.52, N 10.07. Analysis value C 54.52, H 6.58, N 11.04.

[0019] Example 10 [Composition of Pro-Tyr-Pro  $NH_2$  (PYP  $NH_2$ )]

WSCD HCl 1.7g (9.0 millimol) was added to Boc-Tyr (Bzl) 3.25g (8.8 millimol), Pro  $NH_2$  1.0g (8.8 millimol), and HOBt 1.2g (8.9 millimol), it reacted and refined like the example 1, and Boc-Tyr(Bzl)-Pro  $NH_2$  was obtained as colorless oily matter. Acid treatment of the obtained hardening-by-drying object was carried out like the example 1, the Boc radical was removed, vacuum concentration of the solvent was carried out, Et<sub>2</sub>O was added, and the crystal was deposited. This crystal was separated and reduced pressure hardening by drying was carried out in the vacuum desiccator. Z-Pro 2.0g (7.9 millimol) and HOBt 1.2g (8.9 millimol) were added to the crystal which hardened by drying, WSCD HCl 1.7g (9.0 millimol) was dropped, it reacted and refined like the example 1, and oily matter was obtained. This is dissolved in a small amount of methanol, and it is sephadex. It applied to LH-20 column (bore [ of 2.6cm ] x die length of 60cm), and was eluted with this solvent. After looking for the target fraction in TLC and bringing together in one, reduced pressure hardening by drying is carried out, and it is the amorphous-like specified substance. 4.9g was obtained. Sephadex after performing the deprotection reaction according this to a hydrogen catalytic reduction method The colorless amorphous-like specified substance which freeze-dries after purification in G-10 column, and gives a single spot (expansion solvent BAPW system  $R_f=0.42$ ) by TLC 2.9g was obtained. The overall yield so far was 87%. Elemental analysis  $C_{19}H_{26}N_4O_4$  MW 377.44 calculated value C 60.95, H 7.60, N14.96. Analysis value C 56.07, H 7.19, N 13.84.

[0020] Example 11 [Composition of N-acetylation of Pro-Tyr-Pro, and C-amidation object (Ac-PYP  $NH_2$ )]

When add N-ASI 1.0g (7 millimol) to PYP  $NH_2$  1.0g (2.6 millimol) obtained in the example 10, react and refine like an example 2, the fractions which give a single spot (expansion solvent BAPW system  $R_f=0.61$ ) mostly by TLC are collected, it condenses and it freeze-dries, it is the colorless amorphous-like specified substance. 1.05g was obtained. The yield so far was 96%. Elemental analysis  $C_{21}H_{28}N_4O_5$  MW 416.48 Calculated value C 60.56, H 6.78, N 13.45. Analysis value C 55.11, H 6.01, N 13.09 [0021] Example 12 [Composition of Tyr-Ser-Pro (YSP)]

Having dissolved in DMF 20ml and stirring Boc-Ser (Bzl) 2.0g (6.8 millimol), ProOBzl HCl 1.6g (6.8 millimol), and HOBt 1.0g (7.5 millimol) under ice-cooling, it was made to react like the approach indicated in the example 1 in addition to WSCD HCl 1.4g (7.3 millimol) suspended in DMF 10ml, and Boc-Ser(Bzl)-ProOBzl was obtained. Perform the deBoc radical reaction by acid treatment like the approach indicated in the example 1, add Z-Tyr (Bzl) 2.6g (6.3 millimol) and HOBt 0.85g (6.3 millimol) to the obtained residue, WSCD HCl 1.3g (6.8 millimol) is dropped, it is made to react like an example 1, the obtained residue is melted to little MeOH, and it is sephadex. LH-20 column refined. The target fraction was looked for in TLC, and were collected, and reduced pressure hardening by drying was carried out. After dissolving this in EtOAc 50ml, 250ml Et<sub>2</sub>O was added and it crystallized by leaving it in a refrigerator. The crystal was separated and reduced pressure hardening by drying was carried out. (2.40g) . The deprotection reaction according this crystal to a hydrogen catalytic reduction method was performed. Sephadex The colorless amorphous-like specified substance which will give a single spot (expansion solvent BAPW system  $R_f=0.30$ ) by TLC if it refines and freeze-dries in G-10 column 1.70g

was obtained. The overall yield so far was 68%. Elemental analysis C<sub>17</sub>H<sub>23</sub>N<sub>3</sub>O<sub>6</sub> MW 365.38  
Calculated value C 56.03, H 6.63, N 15.37. Analysis value C 55.91, H 6.81, N 13.82.

[0022] Example 13 [Composition of N-acetylation object (Ac-YSP) of Tyr-Ser-Pro]

N-ASI 1.1g (8 millimol) was added to YSP 1.0g (YSP 2.7 millimol) obtained in the example 12, and it was made to react at a room temperature for 2 hours (pH 8 [ about ]). Sephadex after condensing reaction mixture The specified substance which refines in G-10 column and gives a single spot (expansion solvent BAPW system R<sub>f</sub>=0.44) by TLC 0.6g was obtained. The overall yield so far was 54%. Elemental analysis C<sub>19</sub>H<sub>25</sub>N<sub>3</sub>O<sub>7</sub> MW 407.41 Calculated value C 56.01, H 6.18, N 10.31. Analysis value C 54.95, H 5.37, N 10.11.

[0023] Example 14 [Composition of Tyr-Ser-Pro NH<sub>2</sub> (YSP NH<sub>2</sub>)]

Added WSCD HCl 2.0g (10 millimol) to Boc-Ser (Bzl) 2.6g (8.8 millimol), Pro NH<sub>2</sub> 1.0g (8.8 millimol), and HOBt 1.30g (9.7 millimol), it was made to react like an example 1, and Boc-Ser(Bzl)-Pro NH<sub>2</sub> was obtained as oily matter. After carrying out acid treatment like the approach which indicated this oily matter in the example 1 and condensing a solvent, Et<sub>2</sub>O was added and precipitation was obtained in the decantation. Reduced pressure hardening by drying of this was carried out, Z-Tyr (Bzl) 3.2g (7.9 millimol) and HOBt 1.2g (8.7 millimol) were added, and WSCD HCl 1.7g (8.8 millimol) suspended in DMF was dropped. It dissolved in little EtOAc after condensing a solvent, Et<sub>2</sub>O was added, and it crystallized by cooling. 3.25g was obtained when reduced pressure drying of the crystal was separated and carried out. When the fraction of the specified substance which performs a deprotection reaction with a hydrogen catalytic reduction method, refines this crystal in G-sephadex 10 column, and gives an almost single spot (expansion solvent BAPW system R<sub>f</sub>=0.44) by TLC is collected and condensed and it freeze-dries, it is a colorless amorphous-like object. 1.7g was obtained. The overall yield so far was 53%. Elemental analysis C<sub>17</sub>H<sub>24</sub>N<sub>4</sub>O<sub>5</sub> MW 364.39 Calculated value C 56.03, H 6.63, N 15.37. Analysis value C 53.91, H 6.71, N 13.82.

[0024] Example 15 [Composition of N-acetylation of Tyr-Ser-Pro, and C-amidation object (Ac-YSP NH<sub>2</sub>)]

N-ASI 1.0g (7 millimol) was added to YSP NH<sub>2</sub> 0.8g (2.2 millimol) obtained in the example 14, and it was made to react like the approach indicated in the example 2, and processed, and the hardening-by-drying object was obtained. However, since mixing of a condensing agent origin object was presumed by this, it dissolved in little water, and when the extract was repeated 5 times by EtOAc 10ml and the water layer was freeze-dried, 0.87g (yield 95%) of colorless amorphous-like objects was obtained by it. This thing gave the single spot (expansion solvent BAPW system R<sub>f</sub>=0.56) by TLC.

[0025] Example 16 [Composition of Glu-Arg-Pro (ERP)]

WSCD HCl 2.1g (11.0 millimol) was added to Boc-Arg (NO<sub>2</sub>) 1/2AcOEt 1/4H<sub>2</sub>O 3.7g (10 millimol), ProOBzl HCl 2.4g (10 millimol), and HOBt 1.5g (11 millimol), and it was made to react like an example 1, and processed, and Boc-Arg(NO<sub>2</sub>)-ProOBzl was obtained as oily matter. Acid treatment of the obtained oily matter was carried out like the example 1. Z-Glu(Bzl) 1.7g (5.0 millimol), HOBt 0.75g (5.7 millimol), and WSCD HCl 1.1g (5.5 millimol) were dropped at the obtained oily matter, and it reacted to it like the example 1. After dissolving the obtained residue in little EtOAc, it crystallized, when Et<sub>2</sub>O was added and it cooled. The crystal was separated, reduced pressure hardening by drying was carried out, and the 2.3g crystal was obtained. It is sephadex about the residue which obtained this crystal by carrying out in the clearance reaction of all guard chambers with the hydrogen catalytic reduction method. G-10 column refines, and the fraction which gives a single spot (expansion solvent BAPW system R<sub>f</sub>=0.007) by TLC is collected and condensed, and it freeze-dries, and is the colorless amorphous-like specified substance. 1.3g was obtained. The overall yield so far was 32%. Elemental analysis C<sub>16</sub>H<sub>28</sub>N<sub>6</sub>O<sub>6</sub> MW 400.44 Calculated value C 47.99, H 7.05, N 20.99. Analysis value C 45.84, H 7.55, N 19.52.

[0026] Example 17 [Composition of N-acetylation object (Ac-ERP) of Glu-Arg-Pro]

It was obtained in the example 16. N-ASI 1.1g (7.1 millimol) was added to ERP 1.1g (2.7 millimol), and it stirred at the room temperature overnight. Sephadex after condensing reaction mixture G-10 A column refines, and each fraction is collected and condensed, and it freeze-dries, and is the colorless amorphous-

like specified substance. 0.80g was obtained. The overall yield so far was 67%. This thing gave the single spot (expansion solvent BAPW system  $R_f=0.16$ ) by TLC. Elemental analysis  $C_{18}H_{30}N_6O_7MW_{442.47}$  Calculated value C48.86, H6.83, N18.99. Analysis value C45.89, H7.03, N18.07.

[0027] Example 18 [Composition of C-amidation object (ERP NH<sub>2</sub>) of Glu-Arg-Pro]  
WSCD HCl 2.1g (11 millimol) was added to Boc-Arg (NO<sub>2</sub>) 1/2AcOEt 1/4H<sub>2</sub>O 3.7g (10.0 millimol), ProOBzl HCl 2.4g (10 millimol), and HOBt 1.49g (11 millimol), it reacted like the example 1, and chloroform was used and processed instead of EtOAc. It became cloudy when EtOAc was added to the residue which carried out vacuum concentration of the chloroform layer. Furthermore Et<sub>2</sub>O was added, the decantation of the gum-like settlings obtained by leaving it overnight at 4 degrees C was carried out, they were obtained, and reduced pressure hardening by drying was carried out. Acid treatment of the obtained hardening-by-drying object was carried out like the example 1, the Boc radical was removed, the settlings produced during the reaction were obtained by the decantation, and reduced pressure hardening by drying was carried out. After having added Z-Glu (Bzl) 1.7g (5.0 millimol) and HOBt 0.75g (5.5 millimol) to this, dropping WSCD HCl 1.1g (5.7 millimol) and processing by reacting like an example 1, the column refined and colorless oily matter was obtained. Sephadex after performing the clearance reaction of all protective groups for this oily matter with a hydrogen catalytic reduction method G-10 column refined, the fractions of a single spot (expansion solvent BAPW system  $R_f=0.21$ ) were mostly collected by TLC, and crystalline residue was obtained. Cold MeOH was added to this, and 0.2g was obtained when the \*\* collection of the crystal was unfolded and carried out. The overall yield so far was 6%. Elemental-analysis  $C_{16}H_{29}N_7O_5 MW_{399.45}$  Calculated value C 48.11, H 7.32, N 24.55. Analysis value C46.61, H6.92, N22.34.

[0028] Example 19 [Composition of Pro-Thr-Trp (PTW)]  
the DMF solution of Boc-ThrOSu 3.7g (12 millimol) and TrpOBzl HCl 3.8g (12 millimol) -- the bottom of ice-cooling stirring, and TEA -- in addition, it was made to react after checking that it is pH 7-8 overnight After treatment was carried out like the example 1, and Boc-Thr-TrpOBzl was obtained as oily matter. Then, acid treatment was carried out like the example 1, Z-Pro 2.8g (11 millimol) was added to the residue which might be removed in the Boc radical, the DMF 10ml solution of DCCD 2.5g (12 millimol) was dropped, and it was made to react at 4 degrees C overnight. Vacuum concentration of the filtrate is carried out the back according to \*\*, settlings are processed like an example 1, and it is an amorphous-like object. 4.21g was obtained. Among these, the residue which removed 2g with the hydrogen catalytic reduction method, and was obtained in the protective group is dissolved in little water, and it is sephadex. The fractions which refine in G-10 column and give a single spot (expansion solvent BAPW system  $R_f=0.33$ ) mostly by TLC were collected, and reduced pressure hardening by drying was carried out. Little MeOH is added to residue, it was crystallized, after cooling, the crystal was separated, it dried and 1.40g was obtained. The overall yield so far was 30%. Elemental analysis  $C_{20}H_{25}N_4O_5 MW_{402.22}$  Calculated value C 59.69, H 6.51, N 13.91. Analysis value C 55.90, H 6.41, N 12.75.

[0029] Example 20 [Composition of Pro-Thr-Trp NH<sub>2</sub> (PTW NH<sub>2</sub>)]  
Boc-ThrOSu 6.0g (19 millimol) is dissolved in DMF 50ml, and it is Trp NH<sub>2</sub> hydrochloride. 4.6g (19 millimol) and HOBt 2.9g (21 millimol) were added with powder, and the stirring dissolution was carried out. After adding TEA under ice-cooling stirring and checking that it is pH 7-8 WSCD HCl 4.1g (21 millimol) The DMF 10ml solution was added and it was made to react at 4 degrees C overnight. Precipitate is \*\*\*\*(ed) and it is filtrate after vacuum concentration. EtOAc 500ml was added and 8.3g (87%) was obtained by processing like an example 1 by making specified substance Boc-Thr-Trp NH<sub>2</sub> into colorless oily matter. This is made to react like an example 1, and it processes, and is oily matter. 7.2g (16 millimol, 98%) was obtained. To then, this Z-Pro 4.1g (16 millimol), HOBt 2.5g (18 millimol), and WSCD HCl 3.5g (18 millimol) were added, and it was made to react like an example 1, it processed, and 7.3g (70%) was obtained by using Z-Pro-Thr-Trp NH<sub>2</sub> as an amorphous-like object. This amorphous-like object Pd/C 1g was added to 2.5g 10%, and the deprotection reaction was performed after a reaction / processing like the example 1. What was obtained is dissolved in little water and it is



sephadex. The specified substance which will consist of a single spot (expansion solvent BAPW system  $R_f=0.47$ ) if it refines and freeze-dries in G-10 column 1.0g was obtained. Elemental analysis  $C_{20}H_{27}N_5O_4$  MW.401.33 Calculated value C 59.80, H 6.78, N 17.46. Analysis value C 57.63, H 6.94, N 16.27.

[0030] Example 21 [Composition of N-acetylation object (Ac-PTW  $NH_2$ ) of Pro-Thr-Trp  $NH_2$ ]  
After adding N-ASI 0.9g (6 millimol) to PTW  $NH_2$  0.95g (2.4 millimol) obtained in the example 20 and making it react at a room temperature overnight, concentration hardening by drying was carried out under reduced pressure. Silica gel which melted with a small amount of chloroform, and was produced by the mixture of chloroform:methanol =30:1 Regular placing is carried out to C-200 (Wako Pure Chem make) column (bore [ of 2.6cm ] x die length of 45cm), and it is chloroform:methanol =30:1 mixture. 400ml, 10:1 mixture Only 300ml and a methanol were made eluted in 500ml, and each fractions (about 10ml) were collected even for fractions 72-75 after the check by TLC. When residue is dissolved in little water and it freeze-dries after concentration hardening by drying, it is a colorless amorphous-like object. 0.8g was obtained. The overall yield so far is 75%, and is \*\*\*\*. This thing gave the single spot (expansion solvent BAPW system  $R_f=0.64$ ) by TLC. Elemental analysis  $C_{22}H_{29}N_5O_3$  MW443.50 Calculated value C 59.58, H 6.59, N 15.79. Analysis value C 56.98, H 6.54, N 15.34. 3g was obtained.

[0031] Example 22 [Composition of thioPro-Thr-Trp (P'TW)]  
the DMF solution of Boc-ThrOSu 3.7g (12 millimol) and TrpOBzl HCl 3.8g (12 millimol) -- the bottom of ice-cooling stirring, and TEA -- in addition, it was made to react after checking that it is pH 7-8 overnight After treatment was carried out like the example 1, and Boc-Thr-TrpOBzl was obtained as oily matter. Then, acid treatment was carried out like the example 1, the Boc radical was removed, Boc-thioPro2.8g (12 millimol) was added to the obtained residue, the DMF 10ml solution of HOBt 1.784g (13.2 millimol) and WSCD HCl 2.53g (13.2 millimol) was dropped, and it was made to react at 4 degrees C overnight. Vacuum concentration of the filtrate is carried out the back according to \*\*, settlings are processed like an example 1, and it is an amorphous-like object. 6.3g was obtained. Cutting of the protective group by HF of Boc-thioPro-Thr-TrpOBzl 6.3g obtained by coupling went as follows. Boc-thioPro-Thr-TrpOBzl 6.3g was equally divided into ten, and HF cut, respectively. That is, Boc-thioPro-Thr-TrpOBzl is taken in a reaction container, and it is thioanisole. 11ml was added and it put on the room temperature for 1 hour. It was made to react, stirring a reaction container by the dry ice-acetone bath and stirring HF 100ml at installation and 0 degree C under cooling for 1 hour. Reduced pressure clearance of the HF was carried out with the stream aspirator, and it was made to dry with a vacuum pump further. The obtained residue was dissolved by 100ml of acetic-acid water solutions 10%, and the thioanisole which washes twice and remains with 50ml diethylether was removed. The acetic-acid water-solution fraction was neutralized, 3.2g of things which carried out concentration hardening by drying was dissolved in little chloroform-methanol mixture (5:1), the silica gel column chromatography refined, and the specified substance was obtained. Yield of 0.6g. TLC (BAPW system  $R_f=0.62$ ) gave the single spot. Elemental analysis  $C_{19}H_{25}N_4O_5S$  MW420.53 Calculated value C 54.22, H 5.99, N 13.35. Analysis value C 55.90, H 6.41, N 12.75.

[0032] Example 23 [Composition of D-Pro-Thr-Trp  $NH_2$  (D-PTW  $NH_2$ : Pro is D object)]  
the DMF solution of Boc-ThrOSu 3.7g (12 millimol) and TrpOBzl HCl 3.8g (12 millimol) -- the bottom of ice-cooling stirring, and TEA -- in addition, it was made to react after checking that it is pH 7-8 overnight After treatment was carried out like the example 1, and Boc-Thr-TrpOBzl was obtained as oily matter. Then, acid treatment was carried out like the example 1, the Boc radical was removed, Z-D-Pro 2.8g (11 millimol) was added to the obtained residue, the DMF 10ml solution of DCCD 2.5g (12 millimol) was dropped, and it was made to react at 4 degrees C overnight. Vacuum concentration of the filtrate is carried out the back according to \*\*, settlings are processed like an example 1, and it is an amorphous-like object. 4.21g was obtained. Among these The fractions which dissolve the residue which removed the protective group with the catalytic reduction method, and was obtained in 2g in little water, refine in G-sephadex 10 column, and give a single spot mostly by TLC were collected, and reduced pressure hardening by drying was carried out. Little MeOH is added to residue, it was crystallized, after cooling, the crystal was separated, it dried and 1.40g (30% of overall yields) was

obtained (BAPW system  $R_f=0.33$ ). Elemental analysis  $C_{20}H_{25}N_4O_5$  MW 402.22 Calculated value C 59.69, H 6.51, N 13.91. Analysis value C 55.90, H 6.41, N 12.75.

[0033] Example 24 [Composition of Glu-D-Arg (E-D-R: Arg is D object)]

Added WSCD HCl 2.1g (11 millimol) to DMF 100ml under the dissolution and ice-cooling, Z-Glu(Bzl) OH 3.4g (10 millimol), D-Arg(NO<sub>2</sub>) OBzl Tos 4.82g (10 millimol), and HOBT 1.5g (11 millimol) were made to react like an example 1, after treatment was performed, and 5.3g (8 millimol) of oily matter of Z-Glu(Bzl)-D-Arg(NO<sub>2</sub>) OBzl was obtained. The deprotection reaction was performed with the catalytic reduction method, G-sephadex 10 column refined this oily matter, the fractions of the specified substance which gives an almost single spot by TLC were collected, and concentration and freeze drying were performed. 1.82g (6 millimol) was obtained as a colorless amorphous-like object (TLC: BAPW system  $R_f=0.58$ ). Elemental analysis  $C_{11}H_{21}N_5O_5$  and MW 303.33 Calculated value C 43.52, H 6.92, N 23.14. Analysis value C 42.98, H 6.88, N 22.76 [0034] Example of a trial 1 [Focus formation inhibition trial]

Put in the NIH 3T3 cell which carried out the transformation to 8 hole love tech chamber (slide glass size) by proto oncogene erbB-2, and consider one hole as control. A sample is added in remnants and seven holes and it cultivates with a carbon-dioxide-gas incubator 5 37-degree C%, and every 24 hours, for five days, a cell proliferation condition (the number of focuses) is observed with an optical microscope, and a part of the (the sample last concentration 250microg/ml) result a photograph of was taken each time is shown in a table 1 (table 1).

[0035]

[A table 1]

表1 フォーカスアッセイ

サンプル (実施例番号) \ 日数	フォーカス数				
	1	2	3	4	5
9	0	0	0	2	6
13	0	1	1	4	5
17	0	0	0	3	17

[0036] Example of a trial 2 [MTT measuring method]

The NIH 3T3 cell which cancerated by various oncogenes to the DME culture medium which contains a cow blood serum 10% was suspended so that it might become with  $5 \times 10^4$  cells / ml, and the 100microl was poured distributively to each well of 96 hole flat bottom plate. Among these, only the culture medium of tales doses was added to three wells, and it considered as electronegative control. This plate was added to 880microg [ /ml ] concentration in 37 degrees C and 5% carbon-dioxide-gas incubator, and for example, water, and the tripeptide and 10micro of its derivative solution I of this invention prepared with organic solvents, such as an acetone, dimethylformamide, dimethyl sulfoxide, and alcohol, were added to each well after culture overnight (80microg [ /ml ] final concentration). MTT of 5mg [ /ml ] concentration after 2-hour culture and 10micro of PBS (-) solutions I were added to all wells in 37 degrees C and 5% carbon-dioxide-gas incubator, and this was cultivated within 37 degrees C and 5% carbon-dioxide-gas incubator for 2 hours. The at-long-intervals alignment of each plate was carried out 3000 rpm for 15 minutes using the cooling conventional centrifuge equipped with a plate electrode holder. The plate was attracted from the centrifuge, ejection and the supernatant liquid of each well were removed, DMSO of 150microl was added, by the mixer, after stirring, it applied to the immuno reader and the absorbance in 540nm was measured (colorimetry). A part of the result is shown in a table 2 (table 2). It is shown among [ F25, PH1, and S59 ] a table 2 that erbB-2, v-src, v-mos, and SV-40 are the



NIH 3T3 cells which cancerated by a c-H-ras proto oncogene, a v-H-ras proto oncogene, a K-H-ras proto oncogene, erbB-2 proto oncogene, the v-src proto oncogene, the v-mos proto oncogene, and SV-40 proto oncogene, respectively.

[0037]

[A table 2]

実施例番号\細胞	F25	PH1	S59	erbB2	v-src	SV40	v-mos
1	+	+	±	+	+	+	++
2	±	+	±	±	+	+	+
3	+	+	+	++	±	+	++
4	++	++	++	++	++	++	++
5	±	±	±	+	+	±	±
6	+	±	±	+	+	±	+
7	±	+	±	+	+	±	+
8	+	+	+	+	+	+	+
9	+	+	+	++	+	±	+
10	±	+		+	+		
11	++	+	+	++	+	++	++
12	+	±	+	+	+	+	+
13	+	+	±	+	+	+	+
14	±	+	+	+	+	±	±
15	+	+	+	+	+	±	++
16	±	+		+	+		
17	+	±	+	±	+	+	++
18	+	+		±	+		
19	+	+	+	++	++	+	+
20	+	+	+	+	+	+	+
21	+	+	+	+	+	+	+
22	++	++	++	++	++	++	++
23	±	+	+	+	±	+	+
24	++	++	++	++	++	++	++

[Translation done.]